JOURNAL OF VIROLOGY, Mar. 1974, p. 614-622

Vol. 13, No. 3
Printed in U.S.A.

Comparison of JC and BK Human Papovaviruses with Simian Virus 40: Restriction Endonuclease Digestion and Gel Electrophoresis of Resultant Fragments

JUNE E. OSBORN, SYLVIA M. ROBERTSON, BILLIE L. PADGETT, GABRIELLE M. ZU RHEIN, DUARD L. WALKER, AND BERNARD WEISBLUM

Departments of Medical Microbiology, Pathology and Pharmacology, University of Wisconsin Medical School, Madison, Wisconsin 53706

Received for publication 19 December 1973

JC virus was found to have a buoyant density of 1.20 g/cm^3 in linear sucrose-D_2O and 1.35 g/cm^3 in cesium chloride isopycnic gradients. DNA extracted either from JC-infected cultures or from gradient-purified virions occupied a dense position relative to linear DNA in cesium chloride/ethidium bromide gradients, and the circular configuration of the extracted DNA was confirmed by electron microscopy, with a measured molecular weight of 2.93×10^6. DNA from BK virus was similarly prepared and compared to JC and to an SV40 DNA standard by digestion with restriction endonuclease preparations from *Haemophilus influenzae*, *Haemophilus parainfluenzae*, and *Escherichia coli*. Digests were electrophoretically analyzed on gradient polyacrylamide slab gels or agarose gels, and the three viruses were found to have distinctly different cleavage patterns by this form of analysis: JC and BK viruses were almost entirely different from SV40 and significantly different from each other. Thus, JC and BK human papovaviruses appear to be discrete new members of the papovavirus group, rather than SV40 variants.

The recognition of papova-like virions in brain sections from patients with progressive multifocal leukoencephalopathy (26, 32, 33) led to the isolation and propagation in primary human fetal glial (PHFG) cell cultures of a new virus of man, referred to as JC virus (19). This agent has characteristics of a papovavirus of the SV40-polyoma subgroup with respect to virion size and morphology (15), and is serologically distinct from SV40 and polyoma (19). Another recently identified human papovavirus, designated BK virus (13), also differs antigenically from SV40 as well as from JC virus (21, 27; unpublished observations). The virions of JC and BK viruses hemagglutinate human group O erythrocytes, and antibodies measured by hemagglutination (HA) inhibition show both viruses to be ubiquitous in the human populations studied (12, 20).

JC virus is highly oncogenic in newborn hamsters, especially when inoculated by the intracranial route (28). Reports concerning oncogenicity of BK virus have not yet appeared. However, both JC and BK have T antigens which are cross-reactive with the T antigen of SV40. Since previously recognized papovaviruses shared no common antigens, this observation raises questions concerning the relationship of these apparently new human papovaviruses to SV40. Furthermore, Weiner et al. (30) isolated papovaviruses from two cases of progressive multifocal leukoencephalopathy which were indistinguishable from SV40 antigenically and in their characteristics in cell culture. These were found to be SV40 variants by Sack et al. (22).

The present studies were therefore undertaken to purify JC virus, and to compare its DNA with that of BK and SV40 viruses by restriction endonuclease digestion and gel electrophoresis of resultant fragments, to test whether the sharing of antigens among the three viruses represents minor genetic relatedness or is indicative of extensive similarity of genome sequences, as is seen with SV40 and its variants (22).

MATERIALS AND METHODS

Cells. PHFG cells were established in culture according to the method of Shein (25), with MEM (International Scientific Industries, Cary, Ill.) and 10% fetal calf serum for growth, and MEM with 3% fetal calf serum for maintenance medium. When experiments required isotopic labeling, maintenance medium was replaced with MEM containing [*methyl-*

^{3}H]thymidine (specific activity > 20 Ci/mmol, New England Nuclear Corp., Boston, Mass.), 4 μCi/ml, and 10% dialyzed fetal calf serum. Labeling of JC virus was accomplished over the final 8 days of incubation; fresh medium was added at 2- to 3-day intervals.

Viruses. JC virus was originally isolated from the brain of a patient with progressive multifocal leukoencephalopathy (19). The pools used in these experiments represented virus that had been passaged approximately 20 times in PHFG cells. Infection of PHFG cells for the production of JC virus pools was initiated by the inoculation of 6-oz (approximately 180 ml) tissue culture flasks with 200 HA units of JC virus per flask. Initial cytopathic effect in spongioblasts could be observed at 16 to 19 days postinoculation, and cultures were harvested when the cytopathic effect was extensive, usually after 25 to 28 days. In production of both labeled and unlabeled pools of virus, initial harvest methods included incubation of the entire culture at 4 C overnight, decanting of supernatant medium, and subsequent release of virus from the cell pellet by serial treatment with: sonication (20 kc/s for 5 min in an MSE Ultrasonic Disintegrator); receptor-destroying enzyme at 37 C overnight (Microbiological Associates); incubation with 0.01% trypsin and 1% sodium deoxycholate for 30 min at 37 C. Treated preparations were then clarified by low-speed centrifugation, and finally virus was pelleted from the supernatant through 20% sucrose (38,000 rpm in a 40 rotor, Spinco model L-2 ultracentrifuge, for 1 h). The resultant virus pellet was then either stored or placed directly on 13-ml linear sucrose-deuterium oxide gradients (10 to 60%) for subsequent purification and study.

Virus concentration was measured either by hemagglutination of human group O erythrocytes (20) or by infectivity as indicated by numbers of specific immunofluorescent foci observed 10 days postinoculation in PHFG cells grown on 12-mm cover glasses. Fluorescent staining of acetone-fixed cultures was by the indirect method. Anti-JC sera were of rabbit origin; and fluorescein-conjugated anti-rabbit globulin was obtained from Hyland Laboratories (Cosa Mesa, Calif.).

BK virus was kindly provided by Sylvia Gardner. It had been previously passaged through Vero cells, and was passaged twice in this laboratory in PHFG cells in preparation of pools for study. Isotopic labeling of BK virus was initiated at 6 days postinoculation, as early cytopathic effect was observed, and cultures were harvested on day 15. Preparation of pools of BK virus was done in a manner exactly comparable to JC virus methods described above.

Purified DNA of form II, extracted from SV40 strain 707, was kindly provided by Daniel Nathans for use as a standard in the restriction endonuclease studies.

PM-2 phage, used as a marker of molecular weight 6×10^6 (11) in electron microscope studies, was kindly supplied by Waclaw Szybalski.

Lambda phage strain b511 DNA, kindly provided by Fred Blattner and Waclaw Szybalski, was used as a reference marker in restriction endonuclease studies.

Buffers and gradients. SET buffer, used for resuspension of virus pellets and preparation of sucrose-D_2O gradients, contained Tris-hydrochloride, pH 7.4, 0.01 M; NaCl, 0.05 M; and EDTA·Na_2, 0.001 M.

ET buffer, used in the preparation of CsCl and CsCl/ethidium bromide gradients, contained Tris-hydrochloride, pH 7.4, 0.01 M; and EDTA·Na_2, 0.001 M.

E buffer contained Tris acetate, pH 8.0, 0.04 M; Na acetate, 0.02 M; and EDTA·Na_2, 0.001 M. This buffer was used in enzymatic digestion studies. On occasion, ethidium bromide was added to a final concentration of 0.5 μg/ml.

Linear sucrose-D_2O gradients, 10 to 60%, 13 ml, were preformed and allowed to equilibrate at 4 C for at least 24 h prior to use.

CsCl gradients were prepared by mixing saturated CsCl solution with a test sample and ET buffer to desired initial density. For separation of circular from linear DNA, ethidium bromide was added to a final concentration of 100 μg/ml. Gradient density measurements were obtained gravimetrically in all instances.

Radioisotope assay. Portions of solutions to be assayed for ^{3}H-thymidine incorporation were coprecipitated with 1% bovine serum albumin in cold 5% trichloroacetic acid. Precipitates were collected on fiber glass filters, dried, immersed in toluene-based scintillation counting fluid, and counted on a Packard Tri-Carb liquid scintillation spectrometer.

DNA extraction and preparation of circular papovavirus DNA. DNA was extracted from infected and uninfected PHFG cultures by the method of Hirt (14). DNA was extracted from purified virus pools with 2% Sarkosyl NL-30 (K & K Labs, Plainview, N.J.). The resultant DNA was then mixed with a solution of CsCl and ethidium bromide to a final density of 1.56 g/cm^3 and dye concentration of 100 μg/ml. The solution was centrifuged to equilibrium at $102,000 \times g$ (40,000 rpm) in a 65 rotor for 40 h at 20 C. Fractions were collected by a peristaltic pump, and portions were weighed to determine density; in the instance of isotopically labeled DNA, portions were analyzed for acid-precipitable radioactivity. In unlabeled preparations, fractions of appropriate density were extracted twice with equal volumes of isoamyl alcohol, and were dialyzed against SET buffer to remove ethidium bromide and CsCl prior to subsequent study.

Electron microscope preparation and technique. The dense, presumably circular, DNA from CsCl/ethidium bromide gradients was collected. Ethidium bromide was extracted with isoamyl alcohol, and fractions were dialyzed. Samples were prepared for electron microscopy as described by Davis et al. (9) by mixing equal volumes of DNA sample and formamide, and by the addition of cytochrome *c* type III (Sigma Chemical Co., St. Louis, Mo.), 100 μg/ml final concentration. PM-2 phage were added as an internal marker. Samples were picked up on 200-mesh carbon-coated grids, shadowed with uranium oxide and examined in a Hitachi model HU-11-B electron microscope. Photographs were taken at an initial magnification of ×10,800.

Restriction endonuclease enzymatic preparation and digestion. Restriction endonuclease enzymes from *Haemophilus influenzae* strain Rd com^{-10}, and from *Haemophilus parainfluenzae* were prepared as described, respectively, by Danna and Nathans (8) and by Sack and Nathans (23), whereas the enzyme from *Escherichia coli* plasmid-bearing B strain was prepared as described by Mulder and Delius (18). The *Haemophilus* strains were generously provided by Sol Goodgal.

The incubation mixture for digestion with *H. influenzae* enzymes, 50 μliters final volume, contained: Tris-hydrochloride, pH 7.5, 0.0066 M; $MgCl_2$, 0.0087 M; NaCl, 0.035 M; DNA, 25 μg/ml; and 5 μliters of enzyme preparation. Samples were incubated for 9 h at 37 C. The enzyme preparation from the com^{-10} strain was compared with a reference preparation of enzyme kindly provided by Daniel Nathans. The two enzyme preparations were found to give indistinguishable cleavage patterns with reference SV40 DNA (8). Limit digestion was ascertained by the similarity of digestion patterns obtained with two levels of enzyme at a relative twofold concentration increment.

The incubation mixture for digestion with *H. parainfluenzae* enzymes, 50 μliters final volume, contained: Tris-hydrochloride, pH 7.4, 0.001 M; $MgCl_2$, 0.005 M; KCl, 0.006 M; beta-mercaptoethanol, 0.001 M; DNA, 25 μg/ml, and 5 μliters of enzyme preparation. Samples were incubated for 3 h at 37 C.

The incubation mixture for digestion with *E. coli* R I enzyme, 50 μliters final volume, contained: Tris-hydrochloride, pH 7.4, 0.09 M; $MgCl_2$, 0.01 M; DNA, 25 μg/ml; and 5 μliters of enzyme preparation. Samples were incubated for 3 h at 37 C.

Analysis of restriction endonuclease fragments. Enzymatic digestion was terminated by addition of 35 μliters of E buffer containing 20% sucrose and 0.02% bromophenol blue as tracking dye. Samples were fractionated by electrophoresis on acrylamide gel (for the *H. influenzae* digestion mixture) or on agarose gel (for the *H. parainfluenzae* and *E. coli* digestion mixtures). The acrylamide slab gel system contained a gradient of acrylamide from 2.5% to 7.5% in E buffer, as described by Allet et al. (2), whereas the agarose fractionations were performed with a concentration of 1.4% agarose in glass tubes 6 mm in diameter. The agarose was prepared in E buffer containing ethidium bromide, 0.5 μg/ml, as described by Sharp et al. (24).

Gels were either 10 cm in length for agarose or 20 cm in length for acrylamide, depending on the fractionation; electrophoresis was carried out by applying 50 V for a length of time sufficient for the tracking dye to reach the end of the gel.

DNA fragments were visualized as fluorescent bands with the aid of a Mineralite lamp (U. V. Products, San Gabriel, Calif.), after soaking the acrylamide gel for 2 h in E buffer containing ethidium bromide, 0.5 μg/ml. For the agarose gels, treatment with ethidium bromide could be omitted if it was present in the buffers and gel during the run.

Photographs were made with the aid of a Polaroid camera (MP-3) and P-57 film (ASA 3000). Fluorescence was excited with a type B-100A UV light source (U. V. Products, San Gabriel, Calif.) and two filters, type 25 red and type 8 yellow, were used to filter out blue light.

RESULTS

Gradient purification of JC virions. Figure 1 shows the distribution of virions in linear 13-ml sucrose-deuterium oxide (10 to 60%) gradients. A single, sharp peak of radioactivity at 1.20 g/cm^3, present only in infected culture extracts, corresponds exactly to peak levels of both infectivity and hemagglutinin analyzed across the same gradient. When a portion of the peak fraction from sucrose-D_2O gradients was subjected to equilibrium sedimentation in CsCl, the labeled virions formed a single peak of radioactivity at 1.35 g/cm^3. This density is slightly higher than that reported for SV40 (4) and polyoma virions (31), which may in part reflect the method of virion extraction.

Extraction of JC virus DNA from infected cells and from purified virions. A useful property of papovaviruses is the characteristic

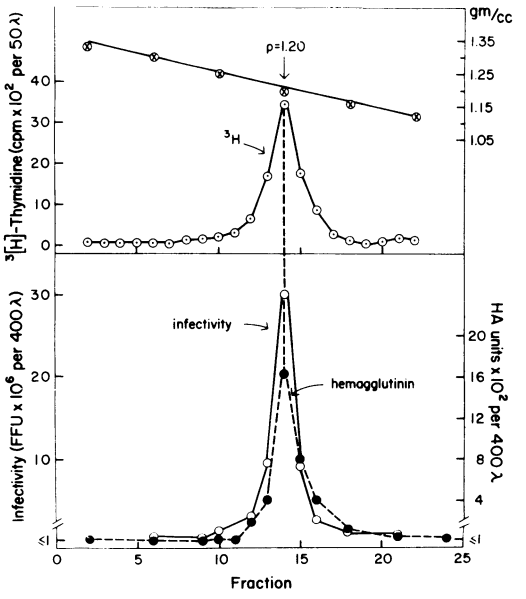

FIG. 1. *Distribution of ^{3}H-thymidine, infectivity, and hemagglutinin of JC virions in an isopycnic sucrose-D_2O gradient (10 to 60%). Virus was pelleted through 20% sucrose, layered on a preformed linear 13-ml sucrose-D_2O gradient, and centrifuged at 201,000 × g for 4 h at 4 C. The gradient was prepared in Tris-hydrochloride, pH 7.4, 0.01 M; NaCl, 0.05 M; and EDTA·Na_2, 0.001 M (SET buffer). Fractions were assayed for acid-precipitable radioactivity, hemagglutinin, and infectivity. FFU, focus-forming units. HA, hemagglutinin.*

circularity of their DNA (6, 10, 29) which allows separation from linear DNA by relative exclusion of dye and resultant greater density in CsCl/ethidium bromide gradients. JC-infected and uninfected PHFG cultures were labeled with ^{3}H-thymidine; DNA was extracted directly from cell monolayers by the method of Hirt (14). After isopycnic centrifugation in CsCl/ethidium bromide gradients, infected cell DNA showed the characteristic presence of a smaller, relatively dense peak of radioactivity at 1.60 g/cm^3 which was absent from DNA of uninfected cultures that had only main band (linear) DNA at 1.55 g/cm^3 (Fig. 2). DNA extracted from gradient-purified virions was similarly analyzed in CsCl/ethidium bromide gradients: as expected, only the denser peak was represented (3, 7).

Determination of JC virus DNA molecular weight by contour length measurement. DNA from purified JC virions, extracted as described above, was further extracted with isoamyl alcohol, dialyzed, mixed with cytochrome *c* (100 μg/ml), formamide (50%) and PM-2 bacteriophage, and then shadowed with uranium oxide and examined on 200-mesh grids by electron microscopy. Figure 3 shows JC circular DNA, which appeared to be approximately half the circumference of PM-2 DNA of known molecular weight 6×10^6. Figure 4 shows a histogram of measurement distribution of 46 JC DNA contour lengths compared with 8 PM-2 circles. The average image contour length for relaxed JC circles was 18.5 ± 1.16 cm, whereas for eight relaxed circles of PM-2, a value of 37.9 ± 0.45 was obtained. Assuming 6.0×10^6 (11) as the average molecular weight of PM-2 DNA, the calculated molecular weight of JC DNA is $2.93 \pm 0.18 \times 10^6$. This value is within the range reported by Crawford and Black (6) for SV40 DNA: 2.7×10^6 by measurement of the band width in a CsCl density gradient and 3.2×10^6 by sedimentation measurements. The obtained value of 2.9×10^6 for JC DNA is likely to be lower than would be obtained if a plaque-purified pool of JC virus were available, since the skew to the left in Fig. 4 probably represents defective forms, as is confirmed by the heterogeneity seen on agarose/ethidium bromide gel electrophoresis described below. Since JC virus cannot, however, be plaqued, this explanation is not presently testable.

Restriction endonuclease digestion and gel electrophoresis of fragments of JC, BK, and SV40 DNAs. BK virus was grown in PHFG cells and purified as described for JC virus, and DNA was extracted from purified virions with 2% Sarkosyl NL-30. ^{3}H-labeled viral DNA

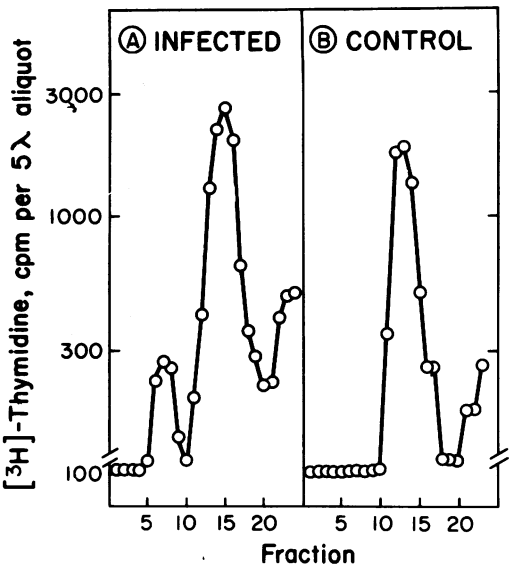

FIG. 2. *Distribution of DNA from infected PHFG cells in cesium chloride/ethidium bromide gradients. ^{3}H-thymidine-labeled DNA was extracted from JC-infected or uninfected control PHFG cells by the method of Hirt (14). The extracted DNA samples were mixed with saturated CsCl to a final density of 1.56 g/cm^3 and ethidium bromide was mixed to a final concentration of 100 μg/ml, total volume 5 ml in ET buffer. Samples were centrifuged in a 65 rotor at 102,000 × g for 40 h at 20 C. Fractions were analyzed for acid-precipitable radioactivity.*

banded in CsCl/ethidium bromide gradients in the same position as that of JC virus DNA. Restriction endonuclease digests of the circular DNA from the two human papovaviruses were then compared with SV40 form II DNA digests.

Figure 5 shows the relative distribution of fragments of SV40, JC, and BK virus DNAs on a gradient polyacrylamide slab gel, after digestion with the *H. influenzae* restriction enzyme preparation. As indicated in the diagram, SV40 fragments are characteristically represented (8), and control studies demonstrated this to be a limit digest. JC and BK DNA fragments are distributed differently from each other in the gel, and show little if any similarity to SV40 in either number or mobility of fragments. Additional weak bands, present in the JC sample and more distinctly in the BK sample, presumably originate from defective forms present in uncloned preparations of other papovaviruses and suggested by both electron microscopy and agarose gel electrophoresis in these experiments.

Further comparison of SV40, JC, and BK virus DNAs was performed with enzymes Hpa and Eco R I followed by fractionation of the

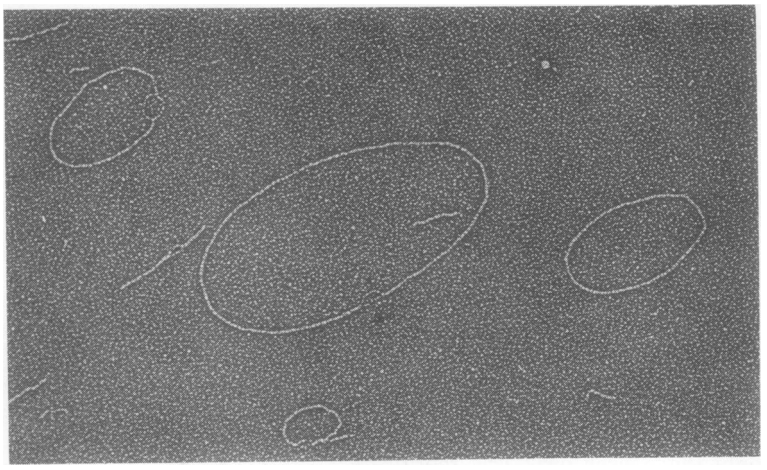

FIG. 3. *Circular DNA extracted from JC virions, compared with PM-2 bacteriophage DNA marker. The dense band from CsCl/ethidium bromide gradients (corresponding to fractions 6 to 8, Fig. 2) was extracted with isoamyl alcohol, dialyzed, and mixed with formamide and cytochrome c, 100 μg/ml final concentration. PM-2 phage were added as internal marker. DNA molecular weight was 6 × 10⁶. Samples were picked up on carbon-coated grids, shadowed with uranium oxide, and examined in a Hitachi model HU-11-B electron microscope. Photographs were taken at an initial magnification of ×10,800. The large circular molecule in the center of the figure represents PM-2 phage DNA; the smaller circles on either side represent JC virus DNA.*

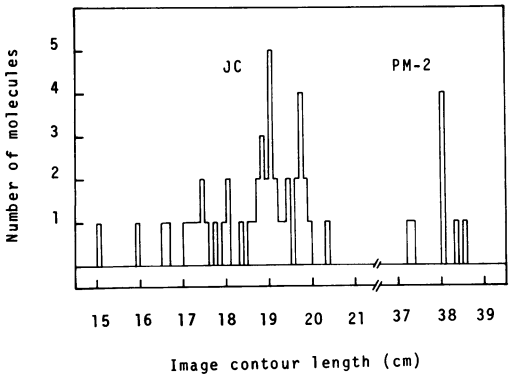

FIG. 4. *Histogram of lengths of circular DNA molecules determined by electron microscopy. Photomicrographs were projected and contour lengths were measured. PM-2 phage DNA, molecular weight 6.0 × 10⁶, was included as internal standard.*

resultant digests on gradient acrylamide gels (Fig. 6). The expected pattern for SV40 described by Sack and Nathans (23) was found; on the other hand, JC and BK showed minimal, if any, degradation by this method and had the same mobility as the undigested SV40 standard. Because of the possibility of resolving forms I, II, and III of SV40 by electrophoresis in agarose/ethidium bromide gels (24) the digestion products obtained with the Hpa enzyme preparation were next examined by this method. Digestion with Eco R I was included for comparison. The results, shown in Fig. 7, reveal the following: undigested SV40 is primarily present as form II, and the main Eco RI digestion product of SV40 is form III, confirming previous work of Morrow and Berg (17), Mulder and Delius (18), Mertz and Davis (16), and Adler and Nathans (1). The undigested JC sample is primarily form I and contains a small amount of form II. Both Hpa bands appear heterogeneous, as would be expected if defective forms are present (5). Both Hpa and Eco RI convert JC to form III. Finally, the undigested BK sample is present primarily as form I, with some form II and III. Again heterogeneity of the DNA in each band is present. Digestion of BK with Hpa results in some increase of form III. Digestion of BK with Eco RI, on the other hand, converts it nearly quantitatively to form III. The mobility of form III identified in these studies is approximately the same as that of the lambda phage fragment 5 which was measured by electron microscopy by Allet et al. (2) and reported to have a molecular weight of 3.0 × 10^6.

DISCUSSION

JC virus was presumed to be a papovavirus on the basis of morphologic and some biologic properties. The present studies confirm and extend the basis for that classification, in that the nucleic acid is demonstrated to be a circular DNA duplex of molecular weight 3 × 10^6, which is within the range of prior reports of SV40 and polyoma DNA (6). Similarly, these studies support the conclusion that BK virus also is a papovavirus with a circular DNA duplex of

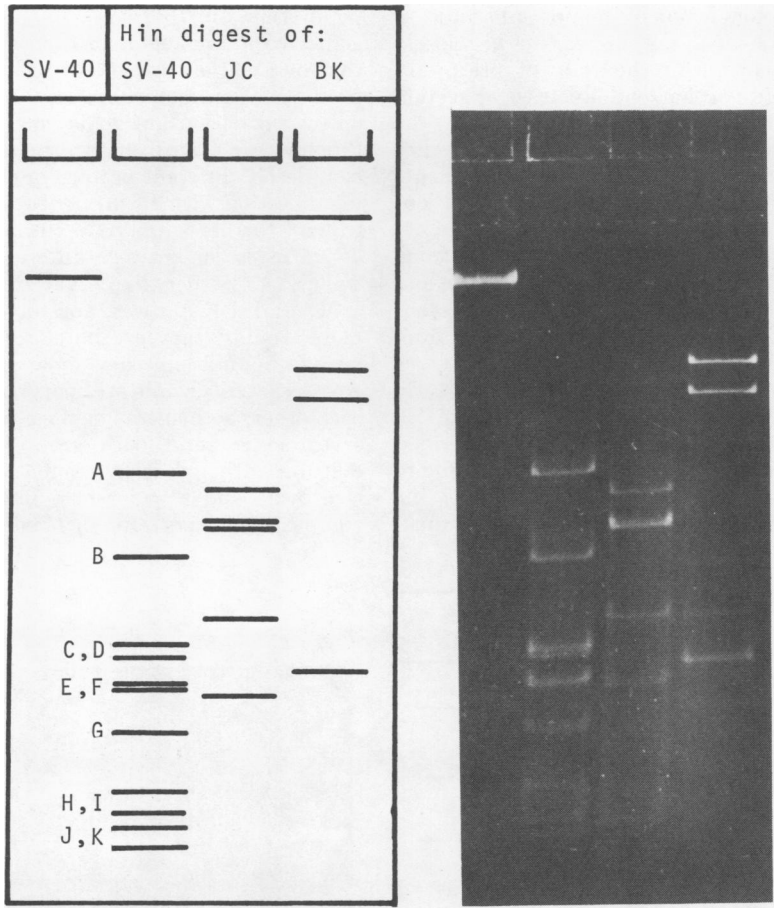

FIG. 5. *Comparison of SV-40, JC and BK fragments produced with the H. influenzae restriction endonuclease preparation by gradient acrylamide slab electrophoresis followed by staining with ethidium bromide. Samples containing 2 μg of DNA were digested with H. influenzae restriction endonuclease and fractionated. The origin is at the top. The complete linear form of SV40 produced by digestion with E. coli R I endonuclease is included in the first slot. The schematic line diagram, shown in the left panel, indicates the positions of the major limit digest fragments found in JC and BK compared with the known fragments, described by Danna and Nathans (8), included as reference standard.*

comparable molecular weight. The distribution of JC circular DNA lengths obtained by image contour length measurement is skewed toward the low side (Fig. 4). As a result, the estimate of molecular weight is probably lower than would be the value for plaque-purified JC virus DNA, in that the most likely reason for the skewed distribution is the accumulation of defective forms in the JC pool. Similar skews have been demonstrated for SV40 when passaged at high multiplicity of infection (5). As no method of plaque production with JC virus has yet been devised, cloning is not presently feasible, so this explanation—although likely—is not presently testable.

The study of JC virus is made complicated by its apparently slow replicative cycle and fastidious host cell requirements which have thus far restricted its cultivation to difficult PHFG cell cultures. These studies of JC virus DNA were greatly facilitated by the application of the technique of ethidium bromide staining of gels during or after electrophoretic separation of DNA fragments, a staining technique initially introduced for such purpose by Sharpe et al. (24). Whereas insufficient radioisotopic labeling could be achieved to facilitate radioautographic studies of JC DNA fragments, the use of ethidium bromide and UV excitation for photographic evaluation of gels permitted study of DNA from unlabeled pools of virus grown under optimal conditions.

The major conclusion of the present studies is that JC virus does not appear to be closely related to SV40, within the limits of interpretation of the restriction endonuclease digestion technique. This is in sharp contrast to the finding of Sack et al. (22) in similar studies performed on two other isolates from patients diagnosed as having progressive multifocal leukoencephalopathy. The two isolates of Weiner et al. (30) consistently demonstrated strong biologic and antigenic similarities to SV40, and by restriction endonuclease analysis were concluded to be SV40 variants, differing from standard SV40 strains only in two or three hypervariable regions of the genome (22). By contrast, JC virus differs markedly from SV40 in biologic characteristics, in that it hemagglutinates human group O erythrocytes, will not grow in primate or other cells than PHFG cells, and is apparently widely spread in the human population. Furthermore, its pattern of oncogenicity in hamsters is distinctly different from that of SV40. JC virus is readily distinguished from SV40 antigenically, and appears to have only weak virion antigenic cross-reactivity, although the T antigens are related. Thus, the completely different pattern of cleavage following digestion with *H. influenzae* enzymes tends to lend further support to the hypothesis that JC virus is an entirely different papovavirus from SV40, rather than a variant.

JC and BK viruses are biologically much more similar: they are both ubiquitous in the human population; they show minor antigenic cross-reactivity while still varying notably from each other; both agents hemagglutinate group O erythrocytes, and both grow well in PHFG cultures, although BK virus has a faster replicative cycle and a wider host range in vitro. A comparison of patterns of oncogenicity awaits

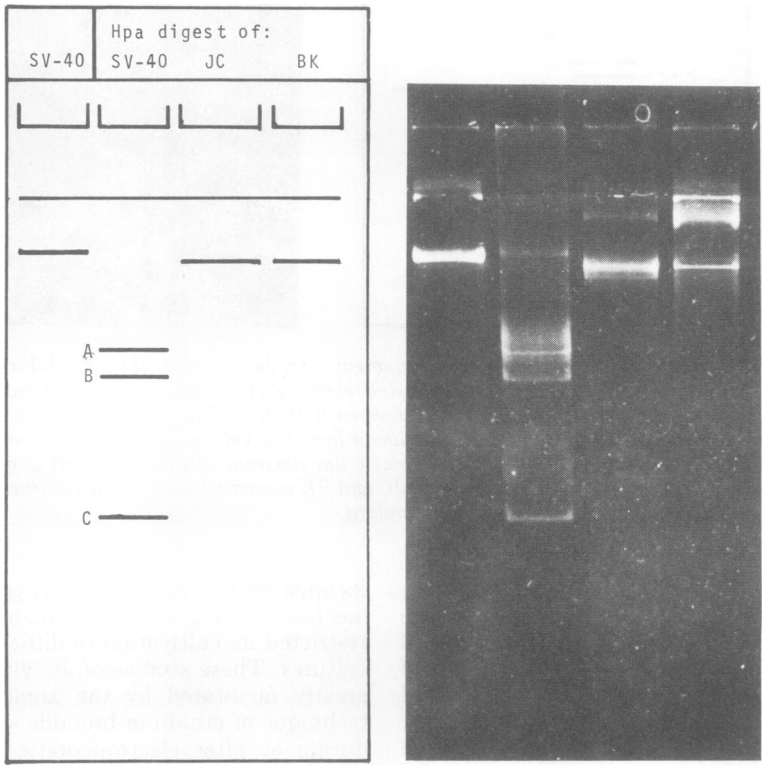

FIG. 6. *Comparison of SV40, JC, and BK fragments produced with the H. parainfluenzae restriction endonuclease preparation by gradient acrylamide slab electrophoresis followed by staining with ethidium bromide. Samples containing 2 μg of DNA were digested with H. parainfluenzae restriction endonuclease and fractionated. The origin is at the top. The complete linear form of SV40 is included in the first slot, as produced by digestion with E. coli R I endonuclease. The schematic line diagram, shown in the left panel, indicates the positions of the major limit digest fragments found in JC and BK compared with the known fragments described by Sack and Nathans (23), included as reference standard.*

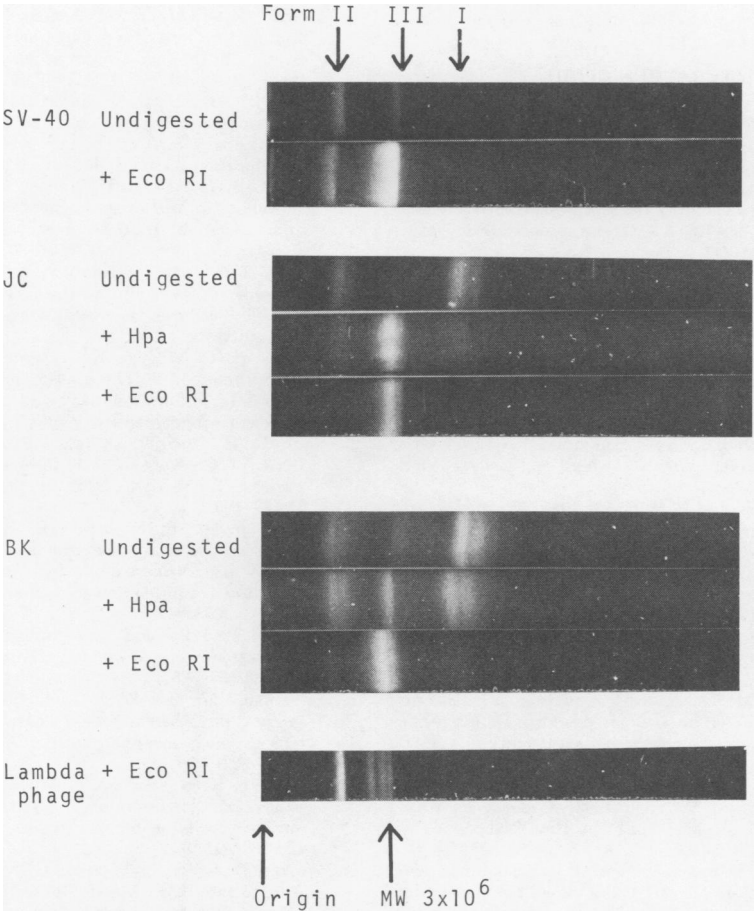

FIG. 7. *Comparison of SV40, JC and BK fragments produced with E. coli R I and H. parainfluenzae restriction endonucleases by agarose gel/ethidium bromide electrophoresis. Samples containing 2 μg of DNA were digested with either E. coli R I or H. parainfluenzae enzymes, and were fractionated. The origin is at the left. The position of forms I, II, and III (super coil, relaxed circle, and linear duplex, respectively) in the gel produced in these digestion mixtures are indicated at the top.*

further reports of BK virus studies. By restriction endonuclease comparison with *H. influenzae* enzyme digestion, JC and BK viruses differ greatly, both in number and size of fragments, from each other as well as from SV40. Mullarkey et al. have recently demonstrated that BK yields Hin fragments totally different from those of SV40 (personal communication), and these findings confirm theirs. BK is only partially converted to form III by Hpa, much of it still remaining as form I, despite the fact that its incubation conditions were identical with those of the JC sample. This finding is probably not due to nonspecific single-stranded breaks because the accumulation of form II, the intermediate that would be expected from such a process, is not seen. By Eco R I digestion, however, JC and BK appear quite similar, in that they both appear to have one cleavage site, as does SV40.

While these comparisons of the two new human papovaviruses, JC and BK, suggest that they are notably different from SV40 and from each other, these findings will be strengthened by molecular hybridization studies, which are currently in progress.

ACKNOWLEDGMENTS

We wish to acknowledge the invaluable advice, encouragement, and generosity of Daniel Nathans in the facilitation of these studies. We also gratefully acknowledge the excellent technical assistance of Doris Metcalf and David Walker, and the help of Michael Fiandt in the preparation of the electron microscope grids and photographs.

This work was supported by Public Health Service grant AI-11217 from the National Institute of Allergy and Infectious

Diseases and by grant NS-11129 from The National Institute of Neurologic Disease and Stroke.

LITERATURE CITED

1. Adler, S. P., and D. Nathans. 1973. Studies of SV 40 DNA. V. Conversion of circular to linear DNA by restriction endonuclease from *Escherichia coli* B. Biochim. Biophys. Acta **299**:177-188.
2. Allet, B., P. G. N. Jeppesen, K. J. Katagiri, and H. Delius. 1973. Mapping the DNA fragments produced by cleavage of λ DNA with endonuclease R I. Nature (London) **241**:120-123.
3. Bauer, W., and J. Vinograd. 1968. The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. J. Mol. Biol. **33**:141-171.
4. Black, P. H., E. M. Crawford, and L. V. Crawford. 1964. The purification of simian virus 40. Virology **24**:381-387.
5. Brockman, W. W., T. N. H. Lee, and D. Nathans. 1973. The evolution of new species of viral DNA during serial passage of simian virus 40 at high multiplicity. Virology **54**:384-397.
6. Crawford, L. V., and P. H. Black. 1964. The nucleic acid of SV 40. Virology **24**:388-392.
7. Crawford, L. V., and M. J. Waring. 1967. Supercoiling of polyoma virus DNA measured by its interaction with ethidium bromide. J. Mol. Biol. **25**:23-30.
8. Danna, K., and D. Nathans. 1971. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Hemophilus influenzae*. Proc. Nat. Acad. Sci. U.S.A. **68**:2913-2917.
9. Davis, R. W., M. Simon, and N. Davidson. 1971. Electron microscope heteroduplex methods for mapping regions of base sequence homology in nucleic acids, p. 413-428. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21 D. Academic Press Inc., New York.
10. Dulbecco, R., and M. Vogt. 1963. Evidence for a ring structure of polyoma virus DNA. Proc. Nat. Acad. Sci. U.S.A. **50**:236-243.
11. Espejo, R. T., E. S. Canelo, and R. L. Sinsheimer. 1969. DNA of bacteriophage PM2: a closed circular double-stranded molecule. Proc. Nat. Acad. Sci. U.S.A. **63**: 1164-1168.
12. Gardner, S. D. 1973. Prevalence in England of antibody to human polyomavirus (B. K.). Brit. Med. J. **1**:77-78.
13. Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme. 1971. New human papovavirus (B. K.) isolated from urine after renal transplantation. Lancet **1**:1253-1257.
14. Hirt, B. 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365-369.
15. Melnick, J. L. 1962. Papova virus group. Science **135**:1128-1130.
16. Mertz, J. E., and R. W. Davis. 1972. Cleavage of DNA by R I restriction endonuclease generates cohesive ends. Proc. Nat. Acad. Sci. U.S.A. **69**:3370-3374.
17. Morrow, J. F., and P. Berg. 1972. Cleavage of simian virus 40 DNA at a unique site by a bacterial restriction enzyme. Proc. Nat. Acad. Sci. U.S.A. **69**:3365-3369.
18. Mulder, C., and H. Delius. 1972. Specificity of the break produced by restriction endonuclease R I in simian virus 40, as revealed by partial denaturation mapping. Proc. Nat. Acad. Sci. U.S.A. **69**:3215-3219.
19. Padgett, B. L., D. L. Walker, G. M. Zu Rhein, R. I. Eckroade, and B. H. Dessel. 1971. Cultivation of a papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet **1**:1257-1260.
20. Padgett, B. L., and D. L. Walker. 1973. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J. Infect. Dis. **127**:467-470.
21. Penny, J. B., and O. Narayan. 1973. Studies of the antigenic relationships of the new human papovaviruses by electron microscopy agglutination. Infect. Immunity **8**:299-300.
22. Sack, G. H., O. Narayan, K. J. Danna, L. P. Weiner, and D. Nathans. 1973. The nucleic acid of an SV40-like virus isolated from a patient with progressive multifocal leukoencephalopathy. Virology **51**:345-370.
23. Sack, G. H., and D. Nathans. 1973. Studies of SV40 DNA. VI. Cleavage of SV40 DNA by restriction endonuclease from *Hemophilus parainfluenzae*. Virology **51**:517-520.
24. Sharpe, P. A., B. Sugden, and J. Sambrook. 1973. Detection of two restriction endonucleases in *Haemophilus parainfluenzae* using analytical agarose-ethidium bromide electrophoresis. Biochemistry **12**:3055-3063.
25. Shein, H. 1965. Propagation of human fetal spongioblasts and astrocytes in dispersed cell cultures. Exp. Cell. Res. **40**:554-569.
26. Silverman, L., and L. J. Rubinstein. 1965. Electron microscopic observations on a case of progressive multifocal leukoencephalopathy. Acta Neuropathol. **5**:215-224.
27. 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.
28. Walker, D. L., B. L. Padgett, G. M. Zu Rhein, A. E. Albert, and R. F. Marsh. 1973. Human papovavirus (JC): induction of brain tumors in hamsters. Science **181**:674-676.
29. Weil, R., and J. Vinograd. 1963. The cyclic helix and cyclic coil forms of polyoma viral DNA. Proc. Nat. Acad. Sci. U.S.A. **50**:730-738.
30. Weiner, L. P., R. M. Herndon, O. Narayan, R. T. Johnson, K. Shah, L. J. Rubinstein, T. J. Preziosi, and F. K. Conley. Isolation of virus related to SV40 from patients with progressive multifocal leukoencephalopathy. N. Engl. J. Med. **286**:385-390.
31. Winocour, E. 1963. Purification of polyoma virus. Virology **19**:158-168.
32. Zu Rhein, G. M. 1969. Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy). Progr. Med. Virol. **11**:185-247.
33. Zu Rhein, G. M., and S. M. Chou. 1965. Particles resembling papovaviruses in human cerebral demyelinating disease. Science **148**:1477-1479.