Comparison of Two Human Papovaviruses with Simian Virus 40 by Structural Protein and Antigenic Analysis

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The proteins of simian virus 40 (SV40) and two human papovaviruses, the hemagglutinating BK virus and the non-hemagglutinating DAR virus, were analyzed and compared by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The virions of SV40 and DAR contain seven proteins. By molecular weight analysis the constituent proteins of SV40 and DAR are identical. Approximately 84% of the viral protein has a molecular weight of 45,000. The major protein of BK virus is 3,000 to 5,000 daltons lighter than the major proteins of SV40 and DAR viruses. The five most rapidly migrating proteins of BK virus are indistinguishable by molecular weight analysis from the corresponding proteins of SV40 and DAR viruses. Radial immunodiffusion and immunoelectrophoresis of whole virus gave lines of identity between SV40 and DAR when reacted with SV40 antibody. SV40 antiserum tested against BK virus and BK antiserum tested against SV40 virus showed no reactivity by complement fixation, immunodiffusion, or immunoelectrophoresis.

Three human papovaviruses have been isolated in cell culture from the brains of patients with the rare demyelinating disease, progressive multifocal leukoencephalopathy (PML). Two of the viruses isolated by Weiner et al. (14) appear to share a close antigenic and biologic relationship with simian virus 40 (SV40). The antigenic and biologic properties of the third virus, JC, isolated by Padgett et al. (5) are still to be elucidated. A fourth papovavirus, which like the JC virus is hemagglutinating, was isolated by Gardner et al. (2) from the urine of a patient who had undergone renal transplantation. This isolate, known as BK virus (BKV), has been shown to possess some antigenic relationship to SV40 (2, 6, 11).

Studies were undertaken to elucidate further the physical and antigenic relationships between two of the human papovaviruses and SV40. The two viruses selected for comparison with SV40 were the hemagglutinating BKV and the non-hemagglutinating virus isolated by Weiner from PML case 2 and referred to herein as DAR. The data presented in this report include a comparative analysis by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis of the proteins of each virus; in addition, results of antigenic analysis of the viruses by complement fixation, immunodiffusion, and immunoelectrophoresis are included.

MATERIALS AND METHODS

Viruses. BKV was the same as that reported previously (11). The virus was propagated in WI-38 human fibroblasts. Small-plaque SV40 was propagated in Vero and CV-1 cells. DAR virus was kindly provided by O. Narayan and was propagated in primary AGMK and CV-1 cells.

Cells. Vero and CV-1 cells were obtained from the American Type Culture Collection. WI-38 cells were used from passage 21 through 30.

Antisera. Antisera to BK and SV40 were obtained from the ascitic fluid of hamsters inoculated with purified virus after the method of Takemoto and Habel (9).

CF tests. The microtiter procedure of Sever (8) was employed in complement fixation (CF) tests.

Virus purification: SV40 and DAR. Cells were grown in Eagle medium plus 10% fetal bovine serum (FBS) in half-gallon roller bottles (Bellco Glass, Inc.). When confluent, bottles were inoculated with 5 to 10 ml of undiluted virus (10^8 PFU/ml) adsorbed for 60 to 90 min at 37 C, and Eagle 5% FBS was added. Cells and supernatant were harvested at 7 to 10 days when cytopathic effect (CPE) was extensive. Clarified supernatant was spun at 25,000 rpm for 3 h in a type 30 rotor, and the pellets were suspended in phosphate-buffered saline (PBS). Cells were treated with 1.3% deoxycholate for 20 min. The lysate and pellet from the supernatant fluid were pooled, layered onto a CsCl cushion ($d = 1.33$), and centrifuged in an SW 25.1 rotor at 23,000 rpm for 2 h. Virus collected from the cushion was banded in CsCl by isopycnic density centrifugation at 35,000 rpm for 16 h in a 50Ti rotor.

BKV. BK virus was purified from infected cells by the method described above. Cells were harvested 2 to 3 weeks after infection. All virus used in these studies had an $A_{260}/A_{280} = 1.40 \pm 0.02$. Virus concentration in milligrams per milliliter was determined by dividing the optical density at 260 by 3.85 after the method of Koch et al. (4).

Preparation of isotope-labeled virus; DAR and SV40. Twenty-four h after inoculation 1 mCi of ^3H -amino acid mixture of 250 μCi of ^{14}C -amino acid mixture, (New England Nuclear Corp.) was added to 60 ml of media.

BK virus. Isotopes at the above concentrations were added to BK infected cells 2 to 3 days after inoculation. Eagle medium supplemented with glutamine was used throughout labeling experiments.

Polyacrylamide gel electrophoresis. Purified virus was disrupted in 8 M urea, 2% mercaptoethanol, and 2% SDS at 44 C for 3 h. Electrophoresis was carried out as described by Estes et al. (1) with the following exceptions. An acrylamide-bisacrylamide ratio of 65:1 was used. Gels stained with Coomassie blue were destained with methanol:acetic acid:water (50:75:875 vol/vol/vol).

Bovine serum albumin (molecular weight 65,000), ovalbumin (molecular weight 45,000), chymotrypsinogen (molecular weight 25,000), equine myoglobin (molecular weight 16,900), and cytochrome c (molecular weight 12,400) were the standards for all molecular weight determinations.

Radioactive gels were fractionated into 0.8-mm slices, dissolved in 0.2 ml of 30% hydrogen peroxide at 80 C overnight, solubilized in an NCS tissue solubilizer (Nuclear Chicago Corp.), and counted in a liquiflor-toluene cocktail.

Immunodiffusion. Radial immunodiffusion was carried out on plates overlaid with a solution of 1 g of agarose dissolved in 50 ml of 0.05 M Barbitol (pH 8.6) plus 50 ml of water. Wells were filled with antigen and antibody and precipitin lines were noted at 28 to 48 h. Plates were soaked for 48 h in PBS, 24 h in distilled water, and were stained with 0.5% amidoschwartz dissolved in 10% glacial acetic acid in methanol. They were destained in 10% glacial acetic acid in methanol and photographed.

Immuno-electrophoresis. Agarose plates of the above composition were loaded with antigen, electrophoresed at 30 mA/plate for 90 min in 0.05 Barbitol (pH 8.6). Plates were stained and photographed after the formation of precipitin lines.

RESULTS

Polyacrylamide gel electrophoresis. Figure 1 illustrates the results of electrophoresis of disrupted virus. SV40 and DAR viruses were indistinguishable and yielded seven distinct bands with molecular weights indicated in Table 1. Electrophoresis of BK virus yielded six proteins. The first and major protein (VP-1) of the BK virus was noted to be 3,000 to 5,000 daltons lighter than the respective VP-1 proteins of SV40 and DAR viruses (Fig. 1).

For convenience, the last five proteins of BK virus, which are seen to have identical molecular weights to the last five proteins of SV40, shall be designated Vp3, Vp4, Vp5, Vp6, and Vp7. No protein of 40,000 daltons (Vp2) was resolvable from the major protein in BK virus.

Coelectrophoresis of ^{14}C -labeled SV40 and ^3H -labeled DAR virus was conducted. As seen in Fig. 2, migration was identical. ^3H -labeled BK virus was then coelectrophoresed with ^{14}C SV40. Vp1 of BK migrated one slice ahead of the Vp1 of SV40 (Fig. 2). Identical results were obtained when ^{14}C -labeled BK virus was coelectrophoresed with ^3H -labeled DAR virus. The second protein band of each virus was less than 1 mm from Vp1 and was therefore not resolvable by the technique used. The 32,000 molecular weight protein was equal to or less than 1% of the total viral protein by isotopic gel electrophoresis, and was frequently not detectable over background.

Composite molecular weights obtained by visual and isotopic procedures are presented in Table 2. The percentage of total protein was calculated from isotopic data.

Inspection of Fig. 1 and 2, and the P values in Table 2 confirm the molecular weight identity of the proteins of DAR and SV40 viruses. The significant observation from these data is that the major protein of BK virus is approximately 3,000 to 5,000 daltons lighter than the major protein of SV40 or DAR virus, whereas the molecular weights of the last five proteins of all three viruses are identical.

Antigenic analysis; CF. Antiserum against SV40 and BKV was tested by CF against known quantities of intact virus. SV40 antiserum titered 1/1,280 against 66 μg of purified SV40. BKV antiserum titered 1/64 against 56 μg of BK virus. BKV antiserum was unable to fix complement when reacted with SV40, and BK antiserum did not fix complement when reacted with SV40. This is in accord with the findings of Gardner et al. (2) that by CF tests SV40 and BKV are not related.

Immunodiffusion. Radial immunodiffusion in agarose was carried out by using the three viruses as antigens against BK and SV40 antisera. Each antigen well was loaded with 10 λ of virus at the following concentrations: BK = 0.57 mg/ml, SV40 = 0.48 mg/ml, DAR = 0.38 mg/ml.

SV40 viral antiserum showed a precipitin reaction and gave lines of identity with SV40 and DAR (Fig. 3). No reaction was noted between SV40 antibody and BK virus (Fig. 3). BK antibody gave precipitin lines with BK

FIG. 1. Electrophoresis on SDS-polyacrylamide gels of DAR virus (1), BK virus (2), and SV40 virus (3).

TABLE 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of SV40, DAR, and BK virus: molecular weights from visual gels

Virus	Vp ₁	Vp ₂	Vp ₃	Vp ₄	Vp ₅	Vp ₆	Vp ₇	No. of deter- minations
SV40	47,375	39,875	37,625	31,750	27,125	18,375	15,100	4
BK	43,250		36,500	31,500	27,250	18,750	15,000	2
DAR	46,833	40,500	36,500	32,000	27,833	19,300	16,500	3

virus but showed no reaction with either SV40 or DAR virus (Fig. 3).

Immunoelectrophoresis. Figure 4 shows the results of simultaneous immunoelectrophoresis of the viruses at pH 8.6 with migration to the anode. Virus concentration was as specified under radial immunodiffusion except as noted. The first and the last antibody wells containing SV40 antiserum yield two arcs on reaction with SV40; a minor leading arc, and a major arc beginning just before the end of the antibody wells. The last antigen well was filled with SV40 virus concentrated twofold prior to electrophoresis to facilitate the appearance of any cross

between BK antiserum and SV40 virus and to enhance the appearance of any minor antigenic determinants of SV40. At this higher virus concentration each of the two arcs noted is seen to have two constituents. No minor or leading arc is seen in the reactions of the SV40 antiserum in the second and third antibody wells with SV40 or DAR. This may be accounted for on the basis of concentration, as DAR was less concentrated than SV40. It is clear that SV40 antiserum shows no reaction with BKV. The arc formed by the reaction of BK antiserum with BKV is distinct in contour and rate of migration from the arcs produced by SV40 and DAR

FIG. 2. Above, coelectrophoresis of ^{14}C -labeled SV40 with 3H -labeled DAR virus. Below, coelectrophoresis of ^{14}C -labeled SV40 with 3H -labeled BK virus.

viruses when they are reacted with SV40 anti-serum. Finally, no reaction between BK anti-serum and SV40 was noted.

DISCUSSION

The molecular weights of the constituent polypeptides of SV40, DAR, and BK viruses have been compared by SDS polyacrylamide gel electrophoresis. SV40 and DAR are indistinguishable, whereas BKV has been shown to

have a lighter major protein. The molecular weights of the five polypeptides of SV40 determined by isotopic gel electrophoresis are slightly greater than those noted by Estes et al. (1), and are in closer accord with the determinations of Girard et al. (3), and Tan and Sokol (2). The two additional polypeptides, VP-2 and VP-4, noted visually on gel electrophoresis most likely are the result of our method of viral disruption.

The immunologic identity of SV40 and DAR

TABLE 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of SV40, DAR, and BK viruses: visual and isotopic data pooled

Virion Poly-peptide	SV40			DAR			BK			P Value	
	Mol wt	N ^a	% ^b	Mol wt	N	%	Mol wt	N	%	SV40/DAR	SV40/BK
Vp ₁ ^c	47,900 ± 1,200 ^c	12	83.6	48,187 ± 1,280	7	83.6	44,778 ± 1,176	9	84.0	>0.05	<0.005
Vp ₂	39,856 ± 860	4		40,500 ± 250	3					>0.05	
Vp ₃ ^c	37,950 ± 1,090 ^c	10	2.7	37,920 ± 1,463	6	1.4	36,500 ± 700	2		>0.05	>0.05
Vp ₄	31,750 ± 646	4		32,000 ± 866	3		32,125 ± 790	2		>0.05	>0.05
Vp ₅ ^c	27,625 ± 694 ^c	11	6.5	27,929 ± 1,134	7	8.4	27,809 ± 1,331	9	8.5	>0.05	>0.05
Vp ₆ ^c	18,508 ± 722 ^c	12	4.7	18,917 ± 970	6	4.2	18,110 ± 775	9	4.4	>0.05	>0.05
Vp ₇ ^c	15,216 ± 940 ⁺	12	6.0	14,943 ± 1,730	7	2.2	14,890 ± 633	9	2.6	>0.05	>0.05

^a N, Number of determinations.^b Percentage of total protein from isotope-labeled virus.^c Molecular weight determined by isotopic and visual gels.

FIG. 3. Radial immunodiffusion with antigens loaded as indicated. The center well in (1) contains SV40 antiserum, and in (2) contains BK antiserum.

reported here is in agreement with previous serologic studies of Penney and Narayan (6). In addition, Sack et al. (7) have analyzed the DNA of DAR utilizing *Haemophilus influenza* restriction endonuclease. Their data support the view that DAR virus, while human in origin, appears to be a variant of SV40.

Our previous work (11) has shown an antigenic relationship between SV40 and BKV detectable by the fluorescent antibody test and the neutralization assay. Gardner et al. (2) and

Penney and Narayan (6) have confirmed an antigenic relationship between SV40 and BKV by using immune electron microscopy. Our current studies utilizing the more conventional and less sensitive immunologic techniques of CF, radial immunodiffusion, and immunoelectrophoresis lead us to conclude that although SV40 and BKV are related, they do possess major antigenic differences.

The importance of this conclusion must be interpreted in light of our observation that

FIG. 4. Immunoelectrophoresis of whole virus. Virus and antibody loaded as indicated. Migration is to the anode. SV40 and DAR give arcs of identity. BK virus gives a unique arc with BK antibody. No heterologous crosses are noted.

SV40, DAR, BK, and JC viruses all induce similar T antigens (11, 13). Since T-antigen is presumably virus specified, as are the viral proteins, our observations regarding SV40 and BKV suggest that regions of homology and difference may exist between the nucleic acids of SV40 and BKV. Studies comparing the nucleic acids of these two viruses are now underway.

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