Comparison of the Serology, Transforming Ability, and Polypeptide Composition of Human Papovaviruses Isolated from Urine

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Four isolates of human papovaviruses (RF, GS, DW, and MG viruses) obtained from the urine specimens of renal allograft recipients in widely separated locations were compared with BK virus. Hemagglutination inhibition tests and plaque neutralization assays showed that all were antigenically related to BK virus. All isolates transformed baby hamster kidney cells, transformation being determined by the ability of the cells to plate in soft agar. Purified preparations of each isolate were iodinated with chloramine T and the polypeptide compositions were compared by electrophoresis of disrupted viruses in polyacrylamide gels containing sodium dodecyl sulfate. The gel patterns of all isolates were similar to that of BK virus. The tryptic digests of two major iodinated virion proteins, VP1 and VP3, were analyzed on an ion exchange column. The peptide patterns of GS, DW, and BK virus were identical; those of RF and MG virus closely resembled the patterns of the above three with only minor differences in some peptides. The results show that the four isolates and BK virus are antigenically closely related, have similar oncogenic potential, and are distinguishable from simian virus 40 and JC virus.

Human papovaviruses, morphologically resembling the rodent polyoma virus and simian virus 40 (SV-40), have been isolated from immunosuppressed individuals. Virus particles have been found in brain tissue of patients with progressive multifocal leukoencephalopathy (PML) who have an impaired immune response because of a disorder of the reticuloendothelial system (22, 36, 38). Other isolations have been made from the urine specimens of kidney transplant patients who were immunosuppressed to prevent graft rejection. After the first isolation by Gardner et al. (7) of a papovavirus (BK) from the urine of such a patient, several other groups have obtained papova-like viruses from similar source material (4, 5, 12, 15, 16, 26).

From studies with the first isolates of these human papovaviruses, three categories were distinguished on the basis of serology of the virions and the restriction enzyme patterns of the viral DNA (21, 23, 31). JC virus, recovered by Padgett et al. (22) from a patient with PML, is different from the PML isolates recovered by

Weiner et al. (36), which appear to be SV-40 variants (27, 37). BK virus is antigenically distinct from the above two types (20, 23), and its DNA shows a different pattern after digestion with restriction endonucleases (10, 21).

In this laboratory we have focused attention on viruses recovered from the urine of kidney transplant patients. Serological surveys have indicated that a large proportion of the adult population (approximately 80%) has antibodies against BK virus (6, 29). No human disease has yet been directly attributed to infection with BK virus, although it has been reported to transform hamster cells (18, 25). Our purpose in commencing the studies described in this article was to determine whether the papovaviruses recovered from renal allograft recipients belong to a single category, i.e., the BK type, or whether they consist of more than one category as do the PML-derived viruses. We have examined five isolates obtained in widely differing geographical locations; two isolates were made by Gardner (6) in England, one was made in South Africa by Lecatsas and Prozesky (14, 15), and two were made in the United States by Dougherty and DiStefano (5) in Syracuse and by us in Chicago.

The serology, transforming ability, and poly-

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peptide composition of the virions, as well as the tryptic peptide patterns of the virion proteins (VPs), suggest that all isolates are related to BK virus and therefore distinct from the other human papovaviruses and SV-40.

MATERIALS AND METHODS

Cells and viruses. BK and its related isolates were grown in cultures of human embryonic kidney cells in the same manner as previously described for this laboratory (18, 39). The cells were grown in Dulbecco modified Eagle medium with 10% fetal calf serum before infection and with 2% fetal calf serum after infection. The designations, the cells used for initial growth, and the origins of the different human papovavirus isolates are listed in Table 1. All were obtained from the urine specimens of renal allograft recipients. BK and GS viruses were isolated by Gardner in London. DW was obtained by us from a screening of 25 kidney transplant patients at this medical center.

To prepare viral stocks, cells were infected at a multiplicity of infection of 0.1 to 1 PFU/cell and harvested 2 to 3 weeks after infection when cytopathic effects were evident. ³H-labeled MG virus was prepared by adding a tritiated L-amino acid mixture (New England Nuclear Corp.) to a final concentration of 10 μ Ci/ml to cell cultures 2 days after infection. ³⁵S-labeled BK virus was grown using a similar protocol with [³⁵S]methionine (New England Nuclear Corp., specific activity of approximately 100 Ci/mmol) at a final concentration of 10 μ Ci/ml. ¹⁴C-labeled SV-40 was prepared as previously described (39). Virus of all strains was purified by first concentrating the virions from cell lysates on a cushion of saturated KBr solution, and then by two successive centrifugations to equilibrium in CsCl solutions (39). All isolates banded in the same region of the density gradient. Viral preparations were dialyzed and stored in Tris buffer (135 mM

NaCl, 5 mM KCl, 0.7 mM Na₂HPO₄, 25 mM Tris[TD]).

Serological analysis of human papovaviruses. The hemagglutination inhibition assay was done in microtiter at pH 6.5 in Alsever buffer with guinea pig erythrocytes. Agglutination was allowed to take place at 4 C for several hours. End points were taken as those dilutions of sera completely inhibiting agglutination by 8 hemagglutination units of virus.

Neutralization experiments were done as plaque neutralization assays (3) with a viral dose of 100 PFU and rabbit anti-BK hyperimmune antiserum. This antiserum had a high titer against BK with little cross-reactivity to SV-40. End points of the plaque neutralization assays were scored as those dilutions of the antisera that neutralized 90% of the viral PFU. Titers for both serological assays were expressed as the reciprocals of the dilution of antisera that inhibited viral activity.

Transformation assays. Viral transformation assays for human papovaviruses were done as previously described using the growth of infected cells in soft agar as the criterion for transformation (18). The experiments followed the protocol of MacPherson and Montagnier (17) in which only baby hamster kidney (BHK) 21 clone 13 cells were used. Virus was allowed to absorb to the BHK cells in suspension for 1 h at 37 C. The cells were suspended in TD at pH 7.2 and not in cell culture medium to reduce fluctuations in pH. After this period, any aggregated cell clumps were disrupted by repeatedly pipetting the virus-cell mixture with a Pasteur pipette drawn out to a capillary. Cells, 2×10^4 in 1.5 ml, were then pipetted onto a 5.0-ml bed of 0.5% agar in Dulbecco modified Eagle medium. The final concentration of soft agar was 0.33% in this medium. Colonies were counted within 16 to 21 days after plating. Usually at this time uninfected BHK cells did not form a background (pinpoint colonies) on control plates.

Iodination and separation of VPs. Purified preparations of virus in TD were iodinated with chloramine

TABLE 1. Sources of human papovaviruses derived from renal transplant patients

Virus	Source	Cells used in isolation	Reference
BK	S. D. Gardner Central Public Health Laboratory London, England	Vero	7
GS	S. D. Gardner Central Public Health Laboratory London, England	Human embryonic lung	
RF	R. M. Dougherty State University of New York Upstate Medical Center Syracuse, N.Y.	Human embryonic kidney	5
MG	G. Lecatsas University of Pretoria Pretoria, South Africa	Human fetal fibroblasts	14, 15
DW	G. Bernhardt and G. di Mayorca University of Illinois at the Medical Center Chicago, Ill.	Human embryonic kidney	

T (9, 39), which preferentially iodinated tyrosine residues. Two-tenths milliliter of viral material with an absorbance at 260 nm of 0.50 was reacted with 500 μ Ci of carrier-free ^{125} I (New England Nuclear Corp.) in the presence of 100 μ g of chloramine T for 10 min at 25 C. The reaction was stopped by the addition of 100 μ g of $\text{Na}_2\text{S}_2\text{O}_5$. The viral preparations were dialyzed against 0.05 M phosphate (pH 7.3), disrupted with 2% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol at 100 C for 2 min, dialyzed, and subjected to electrophoresis in 10% acrylamide gels containing SDS as previously described (39).

Tryptic digests. The method of preparing tryptic peptides was a modification of earlier procedures used by Jacobson et al. (11) and Wright and di Mayorca (39). The iodinated VP was eluted from gel slices into 5 ml of 0.02 M phosphate (pH 7.3) and 0.1% SDS. Ovalbumin (5 mg) was added, and the mixture was passed through a membrane filter (Millipore GSWP) before being concentrated to 1 ml in a dialysis bag with Sephadex G-150. The preparation was dialyzed against 0.1 M Tris (pH 8.5) and 1% SDS, 20 mg of dithiothreitol was added, and then the mixture was incubated at 37 C for 1 h. The preparation was next treated with iodoacetamide at a final concentration of 0.2 M for 3.5 h at 25 C, dialyzed against 0.01 M phosphate (pH 7.3) and 0.05% SDS, concentrated to a volume of 0.2 ml, and dialyzed against 0.05 M NH_4HCO_3 , pH 8.0. Digestion with 0.1 mg of trypsin (treated with TRCK, Worthington) was at 37 C for 22 h, followed by further digestion with another 0.1 mg of trypsin for 3 h. Glacial acetic acid (one-twentieth volume) was added, the total volume was adjusted to 3 ml with 0.2 M pyridine-acetate (pH 3.1), and the preparation was filtered (Millipore GSWP) before a sample was analyzed on a column of Technicon type P chromobeads ion exchange resin maintained at 50 C. The column was developed under pressure with a linear gradient of 0.2 M pyridine-acetate, pH 3.1 (100 ml), to 2 M pyridine-acetate, pH 5.0 (100 ml), at a flow rate of approximately 16 ml/h. In the experiments comparing BK with RF virus, fractions were collected at intervals of 5 min, and in all other column runs, fractions of constant volume (just under 1 ml) were collected. Samples were counted in 5 ml of dioxane-based scintillation fluid.

In the diagrams, the molarity at which each peptide eluted from the column was estimated using the linearity of the elution gradient. This is marked adjacent to the corresponding peak of radioactivity. In the BK and RF comparisons, peptides with the same molarity of elution were not necessarily always at similar fraction numbers. Because the flow rate varied slightly from one column run to another, and the collection of fractions was based on fixed time intervals, a different number of total fractions was obtained in each run. In the other comparisons (fixed-volume collection), peptides with similar molarities of elution were in fractions with similar numbers.

It was difficult to accurately determine the proportion of radioactivity recovered from the column during an experiment because of the quenching caused by the elution gradient. However, assuming that the quenching effect of pyridine-acetate increased uniformly with concentration, we estimated that 90% of

the radioactivity in each initial sample was recovered from the column.

RESULTS

Antigenic similarities of human papovaviruses. Unlike the simian papovavirus SV-40, the human papovaviruses isolated from the urine of renal transplant patients are capable of agglutinating human type O or guinea pig erythrocytes (5, 7). The JC isolate, associated with PML, also hemmagglutinates (22). Therefore we were able to compare the antigenic relationship of human papovaviruses with antisera directed against BK, RF, and JC viruses as well as against SV-40. The data are summarized in Table 2.

The results indicated that BK, RF, GS, DW, and, to a lesser extent, MG virus are antigenically related. First, the antisera against either BK or RF virus cross-reacted directly with BK, GS, RF, and DW viruses, and to a lesser degree with MG virus. Second, SV-40 antiserum did not react appreciably with any of the isolates. Third, the JC antiserum did not react with any of the other human papovaviruses. The reactivity of the JC antiserum with JC virus was 1:5,000 to 1:10,000. The lack of cross-reactivity between BK and JC virus has already been reported (23).

Further serological comparisons were made using virus neutralization as a more accurate and sensitive measure for detecting antigenic relationships between the viruses. The data, shown in Table 3, were obtained from plaque neutralization assays done by the method of Casals (3). Normal rabbit or horse serum showed only borderline activity (titer, less than 20) against SV-40 and BK virus. It is clear that the RF, GS, and DW viruses are closely related to BK virus. The slight cross-reactivity of BK

TABLE 2. Serological comparison of human papovaviruses by hemagglutination inhibition

Serum tested ^a	Virus				
	BK	GS	RF	MG	DW
Anti-BK ^b	5,120	5,120	10,240	320	5,120
Anti-RF ^c	8,000	8,000	8,000	500	8,000
Anti-JC ^d	20	20	20	20	40
Anti-SV-40 ^e	40	40	40	80	80

^a KIO₄ treated.

^b Hyperimmune rabbit antiserum prepared against purified virus.

^c Gift of R. M. Dougherty.

^d Gift of B. L. Padgett.

^e Horse antiserum against SV-40 "V" antigens (Flow Laboratories).

TABLE 3. Serological comparison of human papovaviruses by plaque neutralization

Virus challenge ^a	Antisera	
	Anti-BK (rabbit hyperimmune)	Anti-SV-40 (horse)
BK	80,000	50
RF	110,000	50
GS	50,000	60
DW	200,000	30
SV40	550	5,000

^a Challenge virus was 100 PFU at each dilution of antiserum. Neutralization titers are expressed as the reciprocals of the dilutions of antisera that reduced the plaque formation of 100 PFU of virus by 90%.

virus with SV-40 was consistent with other reports (7, 23, 31).

Transformation by human papovaviruses. BK virus has been shown to transform BHK 21 clone 13 cells (18) and more recently has been shown capable of inducing a fibrosarcoma in a juvenile hamster (28). We began experiments to determine whether other human papovaviruses, isolated from the same source material, also carried the genetic information for cellular transformation. The experiments were carried out as previously described, using growth of infected cells in soft agar as the criterion for transformation. In earlier experiments with BK virus and BHK 21 clone 13 cells, a linear relationship was demonstrated between the multiplicity of infection (from 1 to approximately 100 PFU/cell) and the transformation frequency (18). Beyond a multiplicity of 100 PFU/cell, the frequency of transformation did not increase appreciably. A plateau was reached at high multiplicities of infection, a finding originally described by MacPherson and Montagnier for polyoma virus (17). Therefore, in these experiments we used as high a multiplicity of infection as possible with the available titered stocks of purified virus to be sure that the transformation efficiency of the various papovaviruses was in that plateau region. The data are presented in Table 4. Although there was a degree of variability in the transformation efficiency among the different isolates, they all produced colonies of BHK 21 clone 13 cells in soft agar after infections. Several transformed clones were analyzed by DNA hybridization, and between four and six integrated viral genomes were found per diploid DNA complement (M. Fiori and G. di Mayorca, unpublished data).

Labeled virus. Representative gel patterns

after electrophoresis of iodinated viral preparations are shown in Fig. 1 and 2. Using the same convention for naming the VPs as before (39), the major virion polypeptide VP1 is approximately at fraction 30 in each diagram, VP3 is approximately at fraction 49, VP2 is the small peak between VP1 and VP3, and VP4 and VP5 are the two polypeptides of smallest molecular weight migrating between fractions 60 and 75. Estimates of the sizes of the virion polypeptides have been given previously (1, 20, 39). The composition of the virions and the sizes of the

TABLE 4. Transformation of BHK 21 clone 13 cells by human papovaviruses

Virus	Multiplicity of infection (PFU/cell)	Efficiency of transformation (%) ^a
BK	900	0.42
RF	270	0.45
GS	1,800	0.79
MG	50	0.13
DW	150	0.30

^a Efficiency of transformation is calculated as: [(number of colonies)/(number of cells plated)] × 100.

Fig. 1. Gel electrophoresis of iodinated BK and RF virus.

FIG. 2. Gel electrophoresis of iodinated GS, DW, and MG viruses.

polypeptides of the five isolates in this study were similar, since each of the four heavily labeled peaks was always at the same position as, or at least within one or two fractions of, its corresponding peak in another viral sample subjected to electrophoresis in parallel. VP2 was absent in the particular preparation of BK virus shown in Fig. 1A, but this was not usually the case (39).

The results of co-electrophoresis of MG virus labeled with the ^3H -labeled amino acid mixture

and SV-40 labeled with ^{14}C -labeled reconstituted protein hydrolysate are shown in Fig. 3. As with BK virus (20, 39), VP1 of MG was smaller than VP1 of SV-40, but unlike BK virus in which VP3 was the same size as VP3 of SV-40, VP3 of MG was also smaller than VP3 of SV-40. Although this size difference was slight (one fraction), the finding was reproducible. Co-electrophoresis of ^3H -labeled MG virus and ^{35}S -labeled BK virus (gels not shown) confirmed the size difference in VP3 between

the two isolates and demonstrated that there was no detectable difference in the size of VP1. As shown in Fig. 3, the histone-like proteins were not well resolved, but there was a small difference in the size of VP2 between MG virus and SV-40.

Tryptic peptides of VP1. To compare the tryptic peptides of the VPs of the five isolates, purified viral preparations were taken two at a time and treated in parallel from the initial iodination to the final preparation of tryptic digests for analysis on the column. This approach reduced the possibility of finding differences caused by variation in technique, and also removed the necessity of having all five preparations of virus purified before starting the analyses. BK virus was compared with GS and

with RF; GS virus was compared with DW and with MG. The methods of comparison were the same as those used earlier (39). To compare a particular polypeptide from two different isolates, three separate analyses of labeled tryptic peptides were completed with the ion exchange column. The polypeptide from each isolate was analyzed alone, followed by a mixture of the polypeptides from the two isolates containing equal amounts of radioactivity from the two sources. Peptides were identified by the molarities at which they eluted from the column and also by the relative heights of the peaks. For each preparation either one-half or, more frequently, the same amount of radioactivity as used in the first two analyses was added to the mixture for the final run.

Fig. 3. Co-electrophoresis of MG virus labeled with ^3H -labeled amino acid mixture and SV-40 labeled with ^{14}C -labeled reconstituted protein hydrolysate.

Fig. 4. Analysis of the tryptic peptides of VP1 obtained from iodinated intact virus. The approximate quantities of radioactivity used are shown in parentheses. (A) GS virus (4×10^5 counts/min), (B) DW virus (4×10^5 counts/min), and (C) mixture of GS virus (4×10^5 counts/min) and DW virus (4×10^5 counts/min). Molarities of elution of the peptides are marked.

In Fig. 4, VP1 of GS virus and VP1 of DW virus are compared. The amounts of radioactivity analyzed are stated in the legend of Fig. 4. There were no major differences between the two isolates. Similarly, no differences were detected between VP1 of GS and BK (columns not shown).

However, there were resolvable differences between VP1 of BK and VP1 of RF (Fig. 5), and between VP1 of GS and VP1 of MG (Fig. 6). RF contained a peptide eluting at 1.26 (Fig. 5B) that was absent from BK, and lacked a peptide eluting at 1.15 that was present in BK (Fig. 5A). MG contained a major peptide at 1.72 and a very minor one at 1.25 (Fig. 6B) that were absent from GS. MG also lacked the peptide at 1.15 (Fig. 5A and 6A) and possessed reduced

amounts of that at 1.43 (Fig. 6B). Thus both RF and MG lacked the peptide at 1.12 to 1.15 present in GS, BK, and DW, and contained an additional one at 1.24 to 1.26. MG had yet another unique peptide at 1.72.

From Fig. 4, 5, and 6 it is apparent that whereas the relative amounts of identical peptides in two isolates iodinated at the same time were remarkably similar (e.g., contrast the A and B portion of each figure), there was variation in these amounts from experiment to experiment (e.g., GS in Fig. 4A and in Fig. 6A). The variation was most noticeable for the peptides eluting between 0.6 and 0.9. The reason for this is unknown, although it may have resulted from variations in the pH of the iodination reaction mixture effected by the addition of different

batches of ^{125}I in NaOH.

Tryptic peptides of VP3. The results of the analyses of VP3 followed the same trend as those for VP1. Pairwise comparisons of GS and DW, as well as GS and BK, revealed no differences between these three isolates (columns not shown), whereas RF and MG were different from the above three. In Fig. 7 the analyses of GS and MG are shown. The background radioactivity was much larger in propor-

tion to the peak heights than in the columns of VP1 digests, and only the reasonably well-resolved peaks have been marked. VP3 of MG contained peptides at 1.12 and 1.64 (Fig. 7B) that were absent from VP3 of GS. The latter possessed peptides at 1.23 and 1.46 (Fig. 7A) that were lacking in the preparation of MG. The peptide at 1.29 in Fig. 7B was also possibly unique to MG as there was a shoulder at fraction 130 in Fig. 7C but none on the side of

Fig. 5. Analysis of the tryptic peptides of VP1 obtained from iodinated intact virus. (A) BK virus (3.2×10^6 counts/min), (B) RF virus (3.2×10^6 counts/min), and (C) mixture of BK virus (1.6×10^6 counts/min) and RF virus (1.6×10^6 counts/min).

Fig. 6. Analysis of the tryptic peptides of VP1 obtained from iodinated intact virus. (A) GS virus (5.4×10^5 counts/min), (B) MG virus (5.4×10^5 counts/min), and (C) mixture of GS virus (5.4×10^5 counts/min) and MG virus (5.4×10^5 counts/min).

the peak at 1.23 in Fig. 7A. The comparison of BK and RF is not shown. The only detectable difference between VP3 of BK (and therefore GS, DW) and VP3 of RF was the presence in the latter of an additional peptide at 1.30. This was possibly the same peptide resolved at 1.29 in the analysis of MG.

Tryptic peptides of VP4 and VP5. There was less interest in these smaller polypeptides since it is likely in papovaviruses that they are host coded (see review, reference 33), whereas VP1 and VP3 are coded by the viral genome. A high degree of similarity between these proteins of BK virus and SV-40 has already been established (39). Thus the only comparison made was between VP4 and VP5 combined of BK, and the same proteins of RF (Fig. 8). The two preparations contained many of the same peptides. Peptides at 0.34 and 0.49 in BK (Fig. 8A) were

absent or reduced in amount in RF; peptides at 0.66, 0.96, and 1.24 in RF (Fig. 8B) were absent or reduced in amount in BK. There were at least 13 other peptides in common.

DISCUSSION

Gel electrophoresis of the iodinated virus preparations, when analyzed in parallel, revealed that the molecular weights of the viral polypeptides of the five isolates were similar. Only MG virus demonstrated co-electrophoresis with BK virus. VP1 was the same size in the two isolates, although VP3 of MG was slightly smaller than that of BK virus. This small difference was not detected by electrophoresis in parallel, and so possibly there were similar minor differences between the other isolates. These variations in size are not necessarily detected in the analyses of tryptic peptides by

our technique. If the amino acid sequence of one polypeptide is the same as that of another polypeptide, apart from the addition of further amino acids on the end(s) of one molecule beyond the common sequence, the extra amino acids are detected only if there is an iodinated tyrosine residue in a tryptic peptide from the additional region.

Published results (1, 20, 39) agree on the finding that VP1 of BK virus is smaller than VP1 of SV-40 but disagree on the size of VP3 of BK virus relative to VP3 of SV-40. This is possibly because different strains of SV-40 have been used for comparisons, and also different proteins have been used as standards to estimate molecular weights. An additional protein of BK virus migrating in gels between VP2 and VP3 has been found in preparations of the virus grown in Vero cells (1), although it was not

found in our preparations (Fig. 1 to 3).

We were most interested in the tryptic digests of VP1, VP2, and VP3 since these are almost certainly viral-coded proteins, whereas as mentioned earlier VP4 and VP5 are probably host coded. VP2 was not analyzed since it contained a relatively low proportion of radioactivity and samples free of VP1 would be difficult to prepare. The tryptic peptides of VP1 and VP3 prepared from iodinated intact virus of three separate isolates of BK, GS, and DW viruses were identical. In VP1, both RF and MG viruses lacked a peptide at 1.12 to 1.15 present in the preceding three isolates, and possessed an extra one at 1.24 to 1.26. MG also contained another peptide at 1.72 to 1.73. In VP3, RF virus contained an additional peptide at 1.30 lacking in the above three isolates. MG virus included this with two more peptides at 1.08 to 1.12 and 1.63

Fig. 7. Analysis of the tryptic peptides of VP3 obtained from iodinated intact virus. (A) GS virus (3.2×10^5 counts/min), (B) MG virus (3.2×10^5 counts/min) and (C) mixture of GS virus (3.2×10^5 counts/min) and MG virus (3.2×10^5 counts/min).

Fig. 8. Analysis of the tryptic peptides of VP4 and VP5 obtained from iodinated intact virus. (A) BK virus (9.6×10^4 counts/min), (B) RF virus (9.6×10^4 counts/min), and (C) mixture of BK virus (4.8×10^4 counts/min) and RF virus (4.8×10^4 counts/min).

to 1.64, although it lacked those at 1.20 to 1.23 and 1.45 to 1.46 present in BK, GS, and DW viruses.

From an analysis of tryptic peptides of iodinated polyoma virus, Gibson (8) concluded that the major VP P2 is coded by the viral genome independently of P3 and P4, the two VPs migrating on gels between P2 and the histone proteins (the region in which VP2 and VP3 of SV-40 and BK virus are found). However, all the peptides found in the smaller polypeptide P4 were found in P3, suggesting that P4 and P3 were coded by a common part of the genome. For BK virus and its related isolates, tryptic digests of VP2 were not ana-

lyzed and thus only VP1 and VP3 could be examined for relatedness. The results were not conclusive. On comparing Fig. 6A with 7A (GS virus), and Fig. 6B with 7B (MG virus), it can be seen that there were peptides with similar molarities of elution in VP1 and VP3, particularly in the 0.38 to 0.45 region. There were also major differences in the peptides at higher molarities of elution beyond fraction 100. A more definitive answer to the question of whether the genes for VP1 and VP3 overlap will be available when all tryptic peptides of the proteins have been analyzed and resolved in more than one way.

Experiments describing the marked differ-

ences between the tryptic peptides of iodinated BK virus and SV-40 have been described, and the limits imposed on the possible number of labeled peptides resolved by the use of iodinated virus have been discussed (39). In those experiments, the tryptic digests of VP1 examined were derived from virus iodinated in 0.05 M sodium phosphate buffer (pH 7.3), the virus preparations having been previously disrupted by overnight dialysis against 0.15 M carbonate-bicarbonate buffer (pH 10.5). In all experiments described in this article, infectious virus in TD buffer was iodinated, and therefore the conformation of the capsid proteins was expected to be different. It was feasible that the altered conformation might change the availability of tyrosine residues for iodination substantially, and therefore the pattern of tryptic peptides. Nevertheless, the tryptic digests of VP1 of BK virus were similar in both preparations. The tryptic digest shown in Fig. 5A (VP1 of BK virus) had at least six peptides (at 0.38, 0.43, 0.66, 0.74, 1.15, 1.44) in common with the earlier data for BK virus (39). In the same manner one can compare VP3 obtained from virus iodinated in TD (GS virus in Fig. 7A) with that derived from virus iodinated in 0.05 M sodium phosphate (pH 7.3) (39). Again, most of the major peptides (at 0.45, 0.72, 1.23, 1.46 in Fig. 7A) were present in both preparations. Thus the changes in the ionic strength and pH of the suspending buffer at the time of iodination did slightly alter the number of peptides resolved in VP1 and VP3 and their degree of labeling, but the overall characteristic pattern was unchanged.

VP1 of SV-40 (39) contained peptides at 1.27 and 1.69 that possibly corresponded to those in VP1 of MG virus at 1.25 and 1.72 (Fig. 6B). However, the former pair were very minor components of the total VP1 mixture of SV-40, whereas the peptide of MG virus at 1.72 in Fig. 6B was a major component. Also in VP3 of SV-40 there was another very minor peptide at 1.60 (39) that possibly corresponded to that in Fig. 7B at 1.64, the most significant peptide in VP3 of MG virus. Despite these three possible common peptides there is no doubt that MG virus is much more like BK virus than it is like SV-40, and that the overall dissimilarity in the iodinated tryptic peptide patterns of SV-40 and the urine-derived viruses remains unchallenged.

When the serological analyses (Tables 2 and 3) and the tryptic peptide patterns of the five isolates are considered together, it is apparent that they constitute a group of closely related human papovaviruses. They can be readily distinguished from the primate SV-40 and JC

virus, representative of perhaps another class of human papovaviruses yet to be consolidated by further isolations. In a study of five urine-derived viruses obtained independently at widely separated locations on three continents, our techniques showed no evidence that some were SV-40 variants, as for the PML viruses (37), or were antigenically closely related to JC virus. It is possible that the human papovavirus of the BK type isolated from the urine of renal transplant patients represents a monotypical widespread infection in the same manner as polyoma virus in mice. However, this statement is not made without reservation because the results with MG virus suggest that it is antigenically further removed from BK than the other viruses. Another serological approach, such as the neutralization kinetic studies used to distinguish herpes simplex types 1 and 2, may reveal the existence of more than one serotype of the urine-derived viruses (24).

Renal transplantation and the accompanying immunosuppressive treatment are not the unique prerequisites for the isolation of human papovaviruses from urine. Three viruses serologically identical to BK virus have been isolated from patients with Wiskott-Aldrich syndrome (32) and therefore possessing defective immune responses (2). Further evidence that the recovery of papovaviruses from the urine accompanies immunological deficiency rather than kidney transplantation was obtained by Reese et al. (26), who examined 32 patients being treated with drugs having immunosuppressive effects. Papovaviruses were detected in the urine of three patients; one patient had received a bone marrow transplant and the other two had received no organ transplant at all. At least two of these viruses were antigenically similar to BK virus. No virus was detected in the urine specimens of a matched control group.

All the urine isolates tested by us transformed BHK 21 cells as determined by growth in soft agar, although with some variation in frequency. At this stage we do not wish to draw any conclusion from this variation, as the transformation assays were not done at the same time with an identical cell population, nor were all the viral stocks grown at a low multiplicity of infection avoiding more than one growth cycle. Thus the proportion of defective particles in these stocks was unknown (13, 30). Although JC virus will induce tumors in newborn hamsters (35), transformation of cells in culture by JC has not been reported. Further observations distinguishing JC and the BK type viruses consist of the types of tumors produced in

newborn hamsters. The former induced malignant gliomas when inoculated intracerebrally and subcutaneously at the same time (35), and the latter induced an undifferentiated fibrosarcoma when inoculated subcutaneously (28). A papovavirus serologically related to BK virus (MMV) was isolated from a brain tumor of a patient with Wiskott-Aldrich syndrome (32), in this case from a reticulum cell sarcoma.

To summarize, we have compared the serology, transforming ability, and virion polypeptide composition of five urine-derived human papovaviruses from differing locations. The properties examined suggest that all are antigenically closely related, have similar oncogenic potential, and are distinguishable from SV-40 and JC virus. It is therefore likely that the other papovaviruses isolated from the urine of immunosuppressed patients (12, 15, 26) belong to this class with BK virus considered as the prototypical virus. These and further isolates should be compared with BK virus to firmly establish either the existence of one serotype of urine-derived papovaviruses with similar transforming abilities (suggested by the above results), or the presence of several serotypes, perhaps with highly variable oncogenicity as is the case for the structurally more complex human adenoviruses (34).

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