Characterization of Human Papovavirus RFV: Comparison with SV40 and BKV

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SUMMARY

Human papovavirus, RFV, isolated from urine of a renal transplant patient was compared with two strains of SV40 and with the prototype human papovavirus, BKV. Neutralization tests showed that RFV and BKV are indistinguishable, while large-plaque (LP) and small-plaque (SP) isolates of SV40 gave a low but significant level of cross-reaction with rabbit or human antiserum against RFV.

DNA reassociation saturation tests using 125I-labelled RFV DNA show that BKV has 88% homology, and SP-SV40 has 29% homology to RFV. We conclude that RFV and BKV are nearly, if not totally, identical and are not SV40 variants.

INTRODUCTION

Recent reports have shown the existence of a group of human papovaviruses isolated either from the brain of patients with progressive multifocal leukoencephalopathy (Padgett et al. 1971; Weiner et al. 1972a, b), or from the urine of some renal transplant patients (Gardner et al. 1971; Dougherty & DiStefano, 1974). These viruses are morphologically similar to SV40 (Weiner et al. 1972a, b). The two renal transplant isolates BKV (Gardner et al. 1971) and RFV (Dougherty & DiStefano, 1974) have properties in common. They both have haemagglutinin, grow better in human than in monkey cells (Takemoto & Mullarkey, 1973; Dougherty & DiStefano, 1974) are oncogenic in hamsters in vivo, and cause in vitro transformation of hamster cells (R. M. Dougherty, unpublished data; Portolani, Barbanti-Brodano & LaPlaca, 1975; Shah, Daniel & Strandberg, 1975). In comparison, SV40 does not possess haemagglutinin and grows best in monkey cells but not very well in human cells. This report further elucidates the immunological and biochemical properties of RFV and compares them to BKV and SV40.

METHODS

Chemicals. The following chemicals were purchased: Na125I (NEN, > 350 mCi/ml); 3H-thymidine (Schwarz Mann, 20 Ci/mmol); hydroxypatite, HAP (Bio-Rad, HTP DNA grade); Na2SO3 (Baker); TiCl3·4H2O (Alfa Chem.); receptor destroying enzyme, RDE (Microbiological Associates, 1000 units/ml); and Sephadex G-50, fine (Pharmacia). Scintillation fluid was 95 g PPO, 9·5 g POPOP per 5 gal of toluene (Fisher). All glassware was treated for over 1 h with a 1% solution of 2,4-dimethyl-dichlorosilane (Calbiochem), rinsed in toluene and methanol and then air dried. Phosphate buffer, PB, was composed of

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equimolar quantities of \( \text{NaH}_2\text{PO}_4 \) and \( \text{Na}_2\text{HPO}_4 \) to give the final molar concentrations indicated.

Buffer solutions used were: TBE composed of Earle's balanced salt solution (EBSS) with 0.01 M-tris-HCl at pH 7.2, and TED which is TBE plus 2% calf serum, and 100 units/ml penicillin and 50 \( \mu \text{g/ml} \) streptomycin. SSC is 0.15 M-NaCl, 0.015 M-sodium citrate.

Cell cultures. Primary human embryo kidney (HEK) cells were purchased from Flow Laboratories and grown in 100 mm Petri dishes with MEM (Eagle, 1959) containing 10% tryptose phosphate broth and 10% foetal calf serum. Cells between the 3rd and 5th passage were used for virus cultivation and assay. The CV-1 strain of African green monkey kidney cells were grown in MEM with 10% foetal calf serum.

RFV and BKV were grown in HEK. For the production of tritium labelled virus the foetal calf serum was reduced to 2% at the time of addition of isotope.

Viruses. The origin of RFV was described earlier (Dougherty & DiStefano, 1974). The BK strain of human papovavirus was obtained from Dr G. DiMayorca. BKV was passaged in our laboratory in HEK cells under the same conditions as described for RFV. This strain of BKV was originally obtained from Dr S. Gardner and was propagated in HEK cells exclusively (Major & DiMayorca, 1973). A large plaque isolate of SV40, isolated from Rh-911 and designated LP-4, was obtained from Dr L. Diamond. Dr N. Muzyczka supplied the small plaque isolate of SV40 virus.

Preparation of RFV. One ml of fifth passage virus stock was diluted in TED and adsorbed on to a confluent monolayer of fourth passage HEK cells to give a multiplicity of infection (m.o.i.) of 0.03 p.f.u./cell. After 2 h at room temperature, 10 ml of medium was added and the cells were incubated at 37 °C with medium changes every 3 days until more than 90% c.p.e. was observed, usually in 10 days. Cells were collected, pelleted, washed once in TBE, and frozen and thawed three times. Cells were broken with a tissue grinder and RDE was added to a final concentration of 100 units/ml. The lysate was incubated overnight in a 37 °C water bath. Virus was extracted from the cellular debris by repeated grinding and washing in TBE until negligible amounts of haemagglutinin material was released. The wash fluids were pooled and virus stored at −70 °C or further concentrated by pelleting in a type 21 rotor at 20000 rev/min for 90 min. The pellet was suspended in TED and stored frozen at −70 °C.

Preparation of BKV. BKV was prepared in the same manner as RFV except that fifth passage HEK cells were infected with a m.o.i. of 0.04 p.f.u./cell. Virus was stored at −70 °C in TBE without concentrating.

Preparation of SV40. LP-SV40 was prepared in CV-1 cells at a m.o.i. of 0.01 p.f.u./cell. Virus dilutions were adsorbed to cells for 2 h at 37 °C in TED. Medium was added and cultures incubated until more than 80% c.p.e. occurred. Cells were collected, suspended in a small volume of medium, and frozen and thawed three times. The cell suspensions were sonicated in a Biosonic sonicator with a microprobe attachment and debris pelleted from solution. The virus containing the supernatant fluid was stored at −70 °C.

Isolation of virus DNA. DNA was isolated from infected cell cultures by the Hirt procedure (Hirt, 1967). Confluent HEK cells in 100 mm Petri dishes were infected with RFV at an m.o.i. of 1 p.f.u./cell. Four h later the inoculum was removed and the culture was fed with 10 ml of medium. At 65 to 70 h post-infection (p.i.) the cells were washed twice with cold PBS and lysed by the addition of 0.9 ml of a solution of 0.01 M-tris-HCl, 0.01 M-EDTA, pH 7.20, 0.6% sodium dodecyl sulphate. After 10 min the viscous lysate was gently scraped into 15 ml Corex centrifuge tubes. A stock solution of 5 M-NaCl was added to give a final concentration of 1 M-NaCl. Tubes were gently inverted to mix the solution and stored at
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4 °C overnight. Solutions were centrifuged in a Sorvall RC-2B, SS34 rotor, at 12 500 rev/min (19000 g) for 30 min. The non-viscous supernatant fluid was carefully removed and ethidium bromide solution was added to give 200 to 300 μg/ml final concentration. CsCl was added to give a solution of refractive index 1.3885, which is a solution of mean density 1.57 g/ml with all the salts that are present. Virus DNA was banded in a type 40 rotor in polyallomer tubes at 36000 rev/min for 72 h at 23 °C. Form I and II DNAs were collected separately. Ethidium bromide was extracted twice with an equal volume of isopropanol. The DNA solution was diluted with 4 vol. of 1/10 SSC. DNA was precipitated overnight with 2 vol. absolute ethanol at -20 °C. DNA was pelleted in a Sorvall SS34 rotor at -20 °C, 15000 rev/min for 30 min, and dried under vacuum. The pellet was suspended in 1 x SSC and RNase A, which had been boiled for 10 min, was added to a final concentration of 100 μg/ml. After 1 h at 37 °C the solution was extracted with chloroform-isooamyl alcohol (24:1) until no protein remained. Virus DNA was then precipitated by the addition of 2 vol. absolute ethanol and pelleted as before. DNA was finally suspended in 1 x SSC and stored at 4 °C.

SV4o and BKV DNA were prepared in the same manner except that SV4o was adsorbed for 2 h at room temperature on to CV-1 cells and infected cultures of CV-1 cells were lysed at 60 to 65 h p.i.

For 3H-labelled DNA, 3H-thymidine was added at 16 h p.i. to a final concentration of 10 μCi/ml and left until the cells were lysed. The sp. act. was 4 x 10⁶ ct/min/μg RFV DNA. The extinction ratio at 260/280 nm was 1.84. Concentrations are expressed as E₂₆₀/ml.

Synthesis of ¹²⁵I-labelled DNA. A detailed description of the procedure for radioactive iodine labelling of DNA will be published separately. In brief, purified form I virus DNA in 1 mM-tris-HCl, pH 7.20, was sheared to fragments of 1.5 x 10⁵ daltons by boiling in 0.3 N-NaOH for 20 min (Sharp, Pettersson & Sambrook, 1974). Solutions were chilled in an ice-water bath, neutralized with 3 N-HCl and diluted with 10 vol. of 0.2 M-sodium acetate, pH 4.0, to a final DNA concentration of 200 μg/ml. Reactions were performed with freshly prepared solutions in a conical tip polyethylene Beem capsule (Ladd Research Industries, size 90) in a total vol. of 10 μl. Two to three μl of Na¹²⁵I (to give 1 mCi) was mixed with 1 μl of a solution of 4 x 10⁻⁴ M-Na₂SO₃ in 0.2 M-sodium acetate, pH 4.0. After 20 min at room temperature, 1 to 5 μl of sheared, denatured DNA was added to give 1 μg total DNA. Reactions were initiated by the addition of 2 μl of a solution of 4 x 10⁻⁴ M-TlCl₃ in 0.2 M-sodium acetate, pH 4.0. After 1 h at 60 °C, non-specifically reacted iodine was dissociated by the addition of 0.2 ml of 0.14 M-PB at 60 °C for 1 h. ¹²⁵I-DNA was then eluted in the void volume of a column of Sephadex G-50, fine (1 x 20 cm) with 1 mM-tris-HCl, pH 7.20. Fractions of 0.4 ml were collected and samples assayed for TCA precipitable counts in a Packard Tri-Carb liquid scintillation counter. Sp. act. ranged from 5 x 10⁶ ct/min/μg to 5 x 10⁷ ct/min/μg assuming 100 % recovery of the DNA in the void volume.

Before use, ¹²⁵I-DNA was heat-denatured in 0.14 M-PB, rapidly cooled to 60 °C and immediately adsorbed to a column of HAP. Single-stranded material was eluted with six-column volumes of 0.14 M-PB at 60 °C. Recovery of material was > 90 %. This material was then used for reassociation studies.

DNA-DNA reassociation kinetics. Reassociation was performed essentially as previously described (Britten & Kohne, 1967; Gelb, Kohne & Martin, 1971). DNA solutions, sheared to 1.5 x 10⁵ single-strand mol. wt. as described above were heat-denatured in low salt solution and quickly cooled at 4 °C. Reactions were started by the addition of 1/10 vol. 4 M-PB and rapidly raising the solution temperature to 68 °C. At intervals samples were removed, chilled, and diluted to 0.14 M-PB with ice-cold water, and samples were analysed
on HAP columns. Luer-lok tip plastic syringes of 3 ml capacity were used as columns in a specially designed water bath maintained at 60 °C. Single-stranded DNA was eluted with nine-column volumes of 0.14 M-PB and double-stranded DNA was eluted with ten-column volumes of 0.4 M-PB at 60 °C. Recovery of DNA was > 99%. Effluents were chilled and DNA was precipitated by the addition of 1/10 vol. 100% TCA along with 50 µg carrier yeast RNA. Samples were collected on membrane filters, washed with 5% TCA, dried, and counted in a Packard Tri-Carb liquid scintillation counter. Maximum levels of reassociation with homologous DNAs were 89 to 93%.

Saturation reassociation. A fixed concentration of 125I-DNA was permitted to reassociate to a Cₜ (Britten & Kohne, 1967) of 1 × 10⁻⁵ mol-s/l at which point only 2 to 3% reassociation had occurred. Simultaneous reactions were run for the same length of time in the presence of increasing amounts of test DNA and the degree of reassociation determined on HAP. In all cases test DNA concentrations were sufficient to reach a plateau level of reassociation with homologous 125I-DNA sequences. After the plateau level was attained there was no additional effect of increasing amounts of test DNA.

Antisera. Antisera against purified whole virions of RFV and SP-SV40 were prepared in rabbits. Crude virus preparations were made as described above. Virus was centrifuged for 4 h at 20 °C in a Beckman SW 25-1 rotor at 25000 rev/min over a cushion of 10 ml of saturated KBr buffered at pH 8.0 with tris-HCl. The virus bands were removed, dialysed against PBS (0.15 M-NaCl buffered with 0.01 M-phosphate buffer, pH 6.8), mixed with 0.34 g/ml of CsCl in 15 ml, and centrifuged for 23 h at 20 °C in an SW 40 rotor at 35000 rev/min. The virus bands were removed and dialysed for 48 h against PBS.

Rabbits were given two inoculations, each composed of 0.5 E₃₀₀ units of purified virus in 1 ml, emulsified with an equal volume of complete Freund's adjuvant. The injections were separated by a 2-week interval, and the animals were bled 7 days after the second inoculation. The antiserum was collected and heated at 56 °C for 30 min.

Human antiserum was selected from a large number of specimens on the basis of a high haemagglutination-inhibition titre against RFV.

Neutralization tests. For neutralization tests, serum and virus were diluted in Earle's BSS containing 2% inactivated (56 °C, 30 min) calf serum and buffered with 0.01 M-tris-HCl at pH 7.2. Virus was diluted to contain 250 to 400 p.f.u./ml and 0.1 ml of undiluted serum or a series of 10-fold serum dilutions were added to 0.9 ml of diluted virus, to give a 10-fold serum dilution series starting at 10⁻¹, in a constant amount of virus. Control mixtures contained non-immune serum of the appropriate species, or no additional serum. The mixtures were incubated at 37 °C for 1 h, then overnight at 4 °C. Plaque assays for residual infectious virus were carried out as described previously (Dougherty & DiStefano, 1974). Monolayers of CV-1 cells were used for SV40 assay, and 5th passage human embryo kidney (HEK) cell monolayers were employed to assay RFV and BKV. Duplicate cell cultures in 60 mm plastic Petri dishes were infected with 0.2 ml each of neutralized or control virus.

To estimate serum potency, the surviving fraction of virus at each serum dilution was derived by dividing the average plaque count per test plate by the average control count. The logarithm of this number, log (V/V₀), was plotted on ordinary graph paper against log serum dilution, and the serum dilution that gave 50% plaque neutralization was estimated by graphic interpolation. Results were expressed as a negative logarithm of that serum dilution, which we call the 50% neutralizing dose (ND₅₀).
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Table 1. Virus neutralization*

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>RFV</th>
<th>BKV</th>
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<th>SP-SV40</th>
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<td>1·2</td>
<td>1·5</td>
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<tr>
<td>Rabbit-anti-SP-SV40† Serum no. 1205A</td>
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<td>2·1</td>
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<tr>
<td>no. 1205B</td>
<td>3·0</td>
<td>2·4</td>
<td>6·2</td>
<td>6·0</td>
</tr>
</tbody>
</table>

* Rabbit or human antisera were diluted in EBSS, mixed with the viruses indicated, and plaque assays performed for residual virus (see Methods).
† Pre-immunization sera from the same rabbits were included as controls. No neutralizing activity against any of the viruses was detected with pre-immune rabbit serum diluted 1/10.
‡ Numbers represent the negative logarithms of the calculated dilution of antiserum resulting in a plaque reduction of 50% (ND50). Control assays contained 50 to 80 plaques per Petri dish.

RESULTS

Antigenic relationships of human and simian papovavirus

Table 1 summarizes the results of plaque neutralization tests with rabbit and human antisera to RFV and rabbit antisera to SV40 against RFV, BKV and SV40 (large and small plaque). The two strains of human papovavirus were essentially identical as judged by cross neutralization with both rabbit hyperimmune serum and human serum. Cross neutralization tests using antiserum against BKV were not done due to the lack of serum. There was a highly significant reciprocal cross reaction between the human and simian papovaviruses. However, antisera were consistently 100 to 1000 times as potent against the homologous virus, suggesting a relatively distant relationship between antigens of the human and simian viruses, consistent with previous reports (Gardner et al. 1971; Penney & Narayan, 1973; Takemoto & Mullarkey, 1973; Dougherty & DiStefano, 1974).

DNA sequence homologies

The extent of sequence homology between RFV, SV40 and BKV DNAs was determined by the effect of each DNA species on the reassociation kinetics of radiolabelled virus DNA. The rate of reassociation for 3H-RFV DNA alone and in the presence of added homologous DNA is shown in Fig. 1. Under our conditions the $C_{ot1/2}$ in the presence of homologous sequences is proportional to the initial concentration of those sequences. In Fig. 1, the presence of homologous DNA sequences causes a shift and decrease in the $C_{ot1/2}$ of labelled DNA in proportion to the total concentration of DNA in solution. The addition of twelve genome copies of unlabelled RFV DNA decreased the $C_{ot1/2}$ of 3H-RFV by 13·3-fold. Subtracting for the contribution of the one 3H-copy of DNA gives a 12·3-fold decrease due to the unlabelled DNA.

Since RFV and BKV were isolated from the urine of two different renal transplant patients, it was important to determine the extent of sequence homology between the two viruses. The hybridization kinetics of 3H-RFV DNA was measured in the presence of 10- and 100-fold excess BKV DNA. Fig. 2 shows that a 10-fold excess of BKV DNA resulted in a shift
Fig. 1. Reassociation kinetics of RFV, ³H-RFV DNA (sp. act. 1·5 × 10⁶ ct/min/E unit, 1 × 10⁻³ E unit/ml) was reassociated alone (△—△) or in the presence of unlabelled RFV DNA (1·2 × 10⁻² E units/ml, ▲—▲) in 0·4 m-PB at 68 °C. At intervals samples were diluted to 0·14 m-PB with H₂O and stored at 4 °C until analysed on HAP columns as described in Methods. C₀t₁ was 6·0 × 10⁻⁴ (△) and 4·5 × 10⁻⁵ (▲) mol-s/l. Each point represents a total of 600 ct/min.

Fig. 2. Reassociation kinetics of ³H-RFv v. BKV. ³H-RFV DNA (sp. act. 1·5 × 10⁶ ct/min/E unit, 2 × 10⁻⁴ E units/ml) was reassociated alone (○—○) or in the presence of either 10 genome copies (2 × 10⁻³ E units/ml, ▲—▲) or 100 genome copies (2 × 10⁻² E units/ml, △—△) of unlabelled BKV DNA. Procedures were as described for Fig. 1. C₀t₁ values were 7·0 × 10⁻⁴ (○), 7·5 × 10⁻⁵ (▲), and 8·5 × 10⁻⁶ (△) mol-s/l. Each point represents a total of 600 ct/min.

in the C₀t₁ of 9·35-fold, corresponding to an 83·5 % homology. With a 100-fold excess the C₀t₁ shifted 82·5-fold corresponding to an 81·5 % homology.

**Saturation hybridization**

Since the reported degree of homology between SV40 and BKV is only 20 % (Howley et al. 1975b), we decided to employ a second method of analysis to measure the homology between SV40 and RFV. The extent of reassociation of any DNA sequence is dependent on its initial concentration and the length of time of the reaction as expressed in the C₀t value. In saturation reassociation the apparent C₀t value of the radiolabelled DNA is held constant. In any one set of experiments both the concentration of labelled DNA and the time of the reaction were held constant such that less than 3 % of the DNA reassociated by itself (Miao & Dougherty, 1976). The effect of added test DNA is used to measure the degree of homo-
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Fig. 3. Saturation reassociation of 125I-RFV DNA. 125I-RFV DNA (sp. act. \(2.6 \times 10^6\) ct/min/E unit, \(1 \times 10^{-5}\) E unit/ml) was reassociated in 0.4 M-PB at 68 °C for 2 h to a Cot value of \(1 \times 10^{-2}\) mol-s/l. The base level of reassociation was 1%. Simultaneous reactions were done in the presence of increasing concentrations of added unlabelled DNAs. All reactions were run for 2 h and analysed as described for Fig. 1. Unlabelled DNAs were RFV (\(\square\)), BKV (\(\bigtriangleup\)), SP-SV40 (\(\triangle\)), and salmon sperm (\(\triangleleft\)). Each point represents a total of 17000 ct/min.

As increasing amounts of test DNA are added to the reaction, all homologous sequences in the radiolabelled DNA will be reassociated. Non-homologous sequences in the labelled DNA will not be affected and will remain single-stranded. When all homologous labelled sequences have been reassociated, a saturation or plateau level will be reached after which no more labelled sequences will be reassociated. The plateau level of reassociation is then a direct measure of the degree of sequence homology between the two DNAs.

In order to perform saturation reassociation it is necessary to have DNA of at least \(5 \times 10^6\) ct/min/µg. Therefore, we developed a system to label virus DNA with 125I. As described in Methods, 125I-DNA was self-annealed, a Cot curve plotted, and compared to standard Cot curves. In all cases the Cot curves for 125I- and 3H-DNA were identical (Miao & Dougherty, 1976). RFV 125I-DNA of sp. act. \(5 \times 10^6\) ct/min/µg was reassociated in the presence of increasing quantities of test DNA until plateau levels were reached. The maximum level of reassociation with homologous RFV DNA was 93%, which was adjusted to give the 100% level of reassociation (Fig. 3). In the presence of salmon sperm DNA the plateau level of reassociation was 1%. Simultaneous reactions in the presence of BKV and SV40 DNA saturated at a level of 88% and 29% reassociation respectively. In all cases saturation levels were attained at a concentration of 0.025 E\(_{260}\)/ml or less of unlabelled DNA. This corresponds to \(2.5 \times 10^5\) genome equivalents of unlabelled virus DNA species relative to 125I-RFV.

DISCUSSION

Cross neutralization tests have shown that RFV and BKV are antigenically identical. However, the more sensitive and precise DNA homology data have revealed subtle differences not evident in cross neutralizations. In this study the extent of DNA-DNA
reassociation was measured by selective binding of double stranded DNA to HAP columns. The conditions employed, 0.14 M-PB at 60 °C, are moderately stringent in that single stranded and poorly reassociated DNA will be eluted as non-reassociated material (Britten, Graham & Neufeld, 1974). However, HAP will not distinguish between perfect duplex molecules and those containing short single strand regions. A molecule consisting of double and short single strand regions will be scored as completely reassociated (Britten & Kohne, 1967). Therefore, the values obtained will be maximal values of the degree of reassociation which, in turn, means that the degree of homology is also a maximum value. For RFV and BKV the maximum degree of homology is 88%. The two human papovaviruses are, therefore, nearly, but not totally, identical. The only measurable biological difference which we have so far detected between RFV and BKV is a somewhat greater oncogenicity of RFV in newborn hamsters. We were able to induce tumours in 50% of outbred newborn hamsters inoculated with 10^6 to 10^7 p.f.u. of RFV (Dougherty, submitted for publication) whereas Shah et al. (1975) under similar conditions, reported only one tumour in 80 test animals inoculated with BKV. In both tests hamsters were obtained from the Lakeview Hamster Colony. The fact that Shah et al. (1975) propagated their stock of BKV in monkey cells (Vero) may account for this difference in oncogenicity. Further experiments using BKV propagated in HEK cells should clarify the situation. The minimal 12% non-homology between RFV and BKV may be very important and future studies will determine whether these sequences are responsible for the apparently higher oncogenicity of RFV. At the present time it would seem that both viruses have the same antigenic determinants but differ in a small (12%) but possibly highly significant fraction of their DNA sequences. Other isolates of BKV-like papovaviruses have been obtained from human urine. In one case an isolate (BKV-MM) was found with DNA which was 94% the size of BKV DNA (Howley et al. 1975a). In the case of RFV, however, we have found a 12% non-homology with BKV and an essentially identical DNA mol. wt. (Dougherty & DiStefano, 1974; Howley et al. 1975b).

RFV shows 29% homology with SP-SV40. This is in agreement with the report of Howley et al. (1975b) who have shown that the same SP-SV40 strain exhibits 20 to 30% sequence homology with BKV. Cross neutralization tests show a distant but detectable antigenic relationship between SP-SV40 and RFV or BKV. The level of cross neutralization of SV40 with antiserum against the human papovaviruses shows an approx. 3 log difference in the neutralizing antibody titres between the human and simian papovaviruses. The fact that there is a detectable cross reaction between RFV and both SV40 strains is not surprising since it has already been reported that even antiserum from rabbits acutely infected with BKV cross reacts with SV40 at low level (Penney & Narayan, 1973). Other studies have demonstrated a cross reaction between the T antigens of the human viruses and SV40 (Takemoto & Mullarkey, 1973; Portolani et al. 1975; Shah et al. 1975). However, by selective removal of the cross reacting components from antisera against SV40 or RFV T antigens we have found that the major serum component is distinct for each virus T antigen (R. M. Dougherty, manuscript submitted). This indicates that there are at least two components of T antigen of which only the minor one is common to RFV and SV40. The 29% homology between SP-SV40 and RFV is equivalent to 8.7 × 10^6 daltons of DNA or 435 amino acids. Since both RFV and SV40 can code for only 3 proteins averaging 500 amino acids each, it should be possible to detect polypeptide similarities between RFV and SP-SV40 easily.

From the data presented here it is evident that the two human papovaviruses, RFV and BKV, are very nearly, but not totally, identical. That one may be a variant of the other is very
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likely. Since the prototype human papovavirus is BKV, our isolate could be designated as BKV-RF. Further work on the characterization of RFV and BKV proteins and the fractionation of RFV DNA with restriction enzymes will show whether or not the 12% non-homology is significant.

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REFERENCES


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