Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients


From the Departments of Pathology, St. Mary’s Hospital, London W2 and Southmead Hospital, Bristol, and the Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London NW9 UK

SUMMARY  Human polyomavirus (BK) was detected in two renal allograft recipients as a result of routine examination of Papanicolaou-stained smears of urinary sediment in the light microscope. Infection with this recently identified virus was confirmed by virus isolation and electron microscopy. The cytological, histological, and ultrastructural changes due to the virus are described, and virus excretion is correlated with the clinical progress of the patients and the pathological findings. The transplant ureters in both patients were found to be ulcerated and stenosed, and virus-infected cells were observed in the ureteric epithelium. We suggest that the administration of high-dose steroids in transplantation may permit active infection with human polyomavirus to occur in ureteric epithelium which has been damaged by ischaemia or inflammation.

Infection with human polyomavirus (BK) was first described by Gardner et al. (1971), who isolated the virus from the urinary tract of a renal allograft recipient. The patient (BK) developed clinical evidence of ureteric obstruction coincident with the virus infection, and at laparotomy a segment of donor ureter was found to be ulcerated and stenosed. Since this initial observation was made, serological studies have shown that reactivation of latent BK virus is common in renal allograft recipients (Coleman et al., 1973b) and polyomavirus particles have been observed in the urine of 44% of transplant patients (Lecatsas et al., 1973); but, despite the frequency of infection, there have been no further case reports of BK virus infection associated with ureteric stenosis or obstruction.

In this paper we report pathological changes in two patients who were found to have active infection with human polyomavirus (BK) after renal transplantation. BK virus infection was suspected when numerous large inclusion-bearing cells were seen in routine Papanicolaou smears of the urinary sediment, and the cytological diagnosis was confirmed by electron microscopy and virus isolation. Examination of postmortem material and surgical specimens revealed that, in both cases, BK virus infection was associated with narrowing, fibrosis, and ulceration of the donor ureter.

Special methods of investigation

CYTOMETRY
Ten millilitres of freshly voided urine were concentrated by centrifugation and the deposit was resuspended in 1 ml of the supernatant urine. Three slides were made from the cell suspension by cytocentrifugation (Shandon-Elliot). Two slides were stained by the Papanicolaou method and the third by methyl green pyronin. The slides were screened by light microscopy for activated lymphocytes, inclusion-bearing cells, renal tubular cells, and casts.

ELECTRON MICROSCOPY
Ten millilitres of urine were concentrated by centrifugation and the deposit was resuspended in 0.5 ml of supernatant placed in a conical polyethylene embedding capsule and centrifuged again. The supernatant in the capsule was discarded, leaving a button of cells in the point of the capsule. A 1% solution of buffered osmium tetroxide was carefully layered on to the cell button and left for 4½ hours at 4°C. The osmium was pipetted off without displacing the cell button and replaced with three changes of distilled water and finally with 70% ethanol. After standing overnight at 4°C the button of cells was dislodged and transferred to a glass tube. After dehydration through graded alcohols the cell button was embedded in an epoxy resin mixture. Sections of 1 µ thickness were cut and stained with toluidine blue for
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EXAMINATION
Sections were cut and stained with uranyl acetate and lead citrate for examination in the electron microscope.

VIRUS ISOLATION AND SEROLOGY
Urine samples were held at 4°C. The specimens were centrifuged at slow speed, and the deposit was resuspended in 2 ml of the supernatant; 0.2 ml of this suspension was inoculated into monolayer cultures of human embryo kidney and human embryonic brain cells. The cultures were then incubated stationary at 37°C. Subcultures were made when cytopathic changes were well advanced. An antigen was prepared and the virus identified by the haemagglutination inhibition (HI) test. Infected cell cultures were frozen and thawed three times and sonicated for two minutes using an MSE Ultrasonic Disintegrator Model 60 W. The virus suspension was then treated with fluorocarbon, homogenized for three minutes, and centrifuged. The supernatant was tested for haemagglutinin using 0·5% human O erythrocytes at 4°C. The HI test was performed as described previously (Gardner et al., 1971) but using 16 units of haemagglutinin. Antisera raised against the human polyomavirus strains BK, JC, SB, MG, and the simian polyomavirus SV40 were included in the test.

Sera obtained from the patients were tested for the presence of BK antibody by HI tests (Gardner, 1973).

Case reports

CASE 1
A woman aged 55 was admitted to the Department of Nephrology, Southmead Hospital, Bristol, in July 1975 with chronic glomerulonephritis, hypertension, secondary hyperparathyroidism, mitral incompetence, and chronic renal failure. She failed to respond to a therapeutic regimen, which included a Givonetti diet, antihypertensive drug therapy, and oral calcium and was accepted for the dialysis/transplant programme. Haemodialysis was started in February 1976 with marked clinical improvement, and subtotal parathyroidectomy was performed at this stage.

By June 1976 difficulty was experienced in obtaining blood access, and the patient was prepared for long-term peritoneal dialysis with the insertion of a Tenckhoff cannula. Within 10 days of this operation she developed streptococcal peritonitis, which responded to gentamicin and flucloxacillin. At this stage it was considered that renal transplantation was the only remaining form of treatment available to the patient, and she was placed in European category I and a successful allograft using a cadaveric kidney was carried out on 8 August 1976.

The kidney functioned well in the immediate postoperative period and the creatinine clearance rose slowly until by the 23rd postoperative day it was 50 ml per minute. Immunosuppression was maintained with 125 mg azathioprine and 60 mg prednisolone daily. Two mild rejection episodes were diagnosed on the 10th and 30th postoperative days and treated with a reducing regimen of high-dose oral prednisolone starting with 200 mg daily. A total dose of 3 g Solu Medrone was administered by intravenous pulse dosage over a period of 36 hours and intravenous heparin was also given. During the early post-transplant period the patient was apyrexial and her leucocyte count was within normal limits or chronically raised except for one sharp drop to 2300/mm³ (2·3 × 10⁹/l) on the 31st postoperative day. The white cell count returned to 5800/mm³ (5·8 × 10⁹/l) within four days.

Despite adequate renal function in the post-transplant period the patient was never really well and complained of severe vertigo. On the 65th postoperative day she collapsed and was admitted as an emergency with clinical evidence of acute generalised peritonitis and septicaemic shock. Resuscitation was successful, and at laparotomy a pelvic abscess was located and drained, but her clinical condition did not improve and she died on the 70th postoperative day.

The following major pathological changes of the patient's own organs were observed at necropsy. The mitral valve was the site of severe calcific stenosis. Large abscesses were seen in the left cerebral hemisphere, the lungs, the head of the pancreas, and the left lateral wall of the upper rectum, which was necrotic and perforated in two places. The peritoneal cavity showed a faecal peritonitis maximal in the pelvis. The patient's kidneys were of normal shape but were the size of walnuts and had irregular cystic surfaces. The cortices were narrowed but the renal pelves appeared normal. No abnormality was noted in ureters or bladder.

Histological examination of all tissues showed a depressed inflammatory reaction. Mucor mycelia were detected in the abscesses in the lung and brain. The appearance of the patient's own kidneys were consistent with end-stage disease of uncertain origin. No histological abnormality of the renal pelves, ureters, and bladder was detected.

The pathological changes in the allograft are described in a later section.

CASE 2
A man aged 50 had a history of renal disease, which could be traced to 1944 when he was discharged from the army with haematuria and albuminuria. He was found to be hypertensive in 1963 and was started on methyldopa. During the next 10 years renal function
gradually deteriorated and he was treated with a Giovanetti diet, antihypertensive drugs, and diuretics until, in October 1973, he was admitted into the care of the Department of Nephrology, Southmead Hospital with uraemia, anaemia, and cardiac failure. The patient was accepted for the chronic haemodialysis programme and his clinical condition greatly improved on intermittent dialysis twice weekly. He remained fit and well on this regimen and was placed on the waiting list for renal transplantation.

On 26 August 1976 renal allotransplantation was performed using a cadaveric kidney. The initial postoperative course was satisfactory, and immunosuppression was maintained using azathioprine 125 mg and prednisolone 60 mg daily. A rejection episode recorded on the eighth postoperative day was treated with a total dose of 3 g methylprednisolone (Solu-Medrone), administered intravenously at 12-hourly intervals in 1 g doses, and intravenous heparin. High-dose oral steroids were not indicated as the serum creatinine started falling after the administration of 3 g methylprednisolone.

A second and third episode of rejection were diagnosed on the 38th and 109th postoperative days. On these occasions the patient received 3 g methylprednisolone in divided doses, as above, and intravenous heparin and was started on a reducing regimen of high-dose oral steroids. The function of the transplant kidney continued to deteriorate, and from the 115th day onwards serum creatinine levels remained raised above 200 μmol/l and the creatinine clearance was 125 ml per minute or less. Graft nephrectomy was performed on the 137th postoperative day and the patient is being maintained on haemodialysis. The pathological findings in the allograft are described in a later section.

Results

The pathological features of BK virus infection in cases 1 and 2 are summarised in the Table.

**Table** Pathological features of BK virus infection

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IBCsinclusion-bearing cells; HI—haemagglutination inhibition antibody; IEM—immune electron microscopy
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brane by a halo, which conferred a typical ‘owl’s eye’ appearance on the cell. In later specimens, many of the cells contained large basophilic structureless nuclei surrounded by a thick irregular nuclear membrane. In a few cells, only the thickened nuclear membrane remained to define the boundaries of the nucleus. It was estimated that in the early specimens there were 10³ urothelial cells per ml urine.

Case 2
Urine samples were examined daily until the 31st postoperative day and then at each subsequent outpatient visit and hospital readmission. A specimen of urine obtained on the 110th postoperative day contained a large number of abnormal cells similar to those in case 1 (Fig. 2). The patient continued to exfoliate inclusion-bearing cells from the 110th day until the transplant nephrectomy 27 days later.

ELECTRON MICROSCOPY

Case 1
Thin sections were prepared from a urine sample obtained on the 61st postoperative day. Abnormal urothelial cell identical with those observed in the Papanicolaou smears were seen in a 1 µ section (Fig. 3). In ultrathin sections of the same specimen, spherical virus particles, average diameter 41-2 nm, which were morphologically identical with members of the polyoma subgroup of the papovavirus group, were observed within the nuclei of the cells (Fig. 4). Some nuclei were filled with discrete virus particles but in others the particles were arranged in loose, crystalline array or short chains (Fig. 5).

Case 2
Electron microscopy of a urine sample obtained on the 113th postoperative day showed numerous papovavirus particles in the nuclei of the cells, as in case 1. The average diameter of the particles was 43 nm (Figs 6 and 7).

VIRUS ISOLATION AND SEROLOGY

Case 1
A papovavirus identified as BK virus was isolated in both human embryo kidney and human embryo brain cell cultures from three urine samples collected on days 51, 59, and 63 after the transplant operation. BK haemagglutination inhibition antibody titre in the patient’s serum was 1:20 on day 50 and 1:640 on day 63. Cold agglutinins were detected in the sera examined.

Case 2
The papovavirus visualised in the patient’s urine was identified as BK virus by Dr A. M. Field using the technique of immune electron microscopy directly on the virus particles in the urine. A polyomavirus was isolated from urine collected on the 113th day and

Fig. 2  Case 2. Papanicolaou-stained smear of urinary sediment showing cytopathic changes similar to those in case 1. × 1250

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Fig. 3  Case 1. 1-μ section of urinary sediment showing virus-infected cells identical with those seen in Papanicolaou-stained smear. Toluidine blue stain × 1250.

Fig. 4  Case 1. Ultrathin section of urinary sediment showing numerous spherical polyomavirus particles, 41-2 nm in diameter, in nucleus of urothelial cell. Uranyl acetate and lead citrate × 7500.
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from tissue fragments from ureter and renal pelvis obtained at graft nephrectomy. All the polyomavirus strains isolated in cell culture from this patient have so far proved untypable by HI tests with specific antisera against the prototype strains. These results suggest an alteration in antigenic structure during passage in cell culture and are being studied further.

BK haemagglutination inhibition antibody titre was 1:20, 480 on the 113th postoperative day after transplantation. A second serum sample obtained on the 117th postoperative day had a titre of 1:10, 240.

GROSS APPEARANCE AND HISTOLOGY OF ALLOGRAFT

Case 1
The transplant kidney was of normal size and shape with a smooth external surface. On section the cortex was pale and a little swollen. The ureter passed through the inflammatory tissue surrounding the pelvic abscess, and here it was stenosed, not admitting a fine probe.

Histological studies of the allograft kidney showed no rejection phenomena, and vessels were patent and showed no active disease. The renal pelvis appeared normal but there was a dense inflammatory infiltrate in the wall of the ureter, especially in its lower half where the inflammatory cells appeared to break up the muscle layers and infiltrate the submucosa. The urothelium of the ureter was detached throughout its length. In the upper half the epithelium was missing completely, while in the lower half clusters of urothelial cells could be seen in the narrowed ureteric lumen. A few of these cells were enlarged, and each contained a basophilic intranuclear inclusion and a greatly thickened nuclear membrane (Fig. 8).

Case 2
The renal allograft showed some scarring at one pole, and the tissue was swollen and pale with haemorrhage and necrosis of the renal pelvis. A segment of ureter removed at graft nephrectomy appeared narrowed and fibroed.

On histological examination the renal cortex of the
Fig 6  Case 2. Ultrathin section of urinary sediment showing numerous discrete polyomavirus particles, 43 nm in diameter, in nucleus of urothelial cell. Uranyl acetate and lead citrate × 11150.

Fig. 7  Case 2. Part of nucleus in Fig. 6 at higher magnification. × 31800
allograft showed changes typical of chronic rejection with marked narrowing of all intralobular arteries due to intimal hyperplasia. The renal pelvis showed areas of necrosis and granulation tissue. In some areas, where the epithelium was intact, a nuclear atypia and occasional basophilic intranuclear inclusions were seen (Fig. 9). A similar appearance has been described by ZuRhein and Varakis (1974) in the kidney of a male patient treated for lymphoma. They reported at necropsy large basophilic nuclei in cells bordering the lumina of the calyces and papillary ducts, and papovavirions, 43 nm in diameter, were observed in ultrathin sections of the nuclei.

A one-inch segment of ureter was examined histologically. One end of the ureter was found to be destroyed by nonspecific granulation tissue extending through all coats, but the other end showed no inflammatory infiltrate. The surface cells of the urothelium contained abnormal large cells with hyperchromatic nuclei and greatly thickened nuclear membranes. Electron microscopy of the ureteric epithelium revealed papovavirions in the nuclei of the urothelial cells (Fig. 10).

Discussion

Cytological examination of urinary sediment can play an important role in screening for human polyomavirus as the conventional methods of virus isolation or electron microscopy are not suitable for this purpose (Coleman et al., 1973b). Isolation of BK and JC viruses in monolayer culture and examination of negatively stained preparations of urine in the electron microscope are both time-consuming procedures and impractical for screening large numbers of patients. It is policy both at Southmead Hospital and at St. Mary's Hospital to screen all patients admitted for renal transplantation for human polyomavirus infection using cytological methods. Daily urine samples are obtained from the patients while in hospital and thereafter at outpatient attendances. Cytology offers a method of detecting new cases of infection and of determining the duration of active infection. The cytological diagnosis can be confirmed by virus isolation or electron microscopy.

The two cases presented here illustrate the value of

Fig. 8 Case 1. Histological section of donor ureter. A cluster of urothelial cells lie in the lumen. One cell contains an intranuclear inclusion, and several have basophilic nuclei surrounded by a thickened nuclear membrane. Haematoxylin and eosin × 700.
this method of investigation. We have found that, by correlating virus excretion with the clinical progress of the patient and the pathological findings, a pattern of morbidity associated with human polyomavirus infection emerges. Both patients were found to exfoliate virus-infected cells continuously for more than three weeks. In both patients the onset of infection was apparent within 48 hours of the administration of large doses of prednisolone and Solu-Medrone for graft rejection, and ureteric stenosis was an unexpected pathological finding.

The narrowing of the ureteric lumen was the result of ulceration and inflammation localised to a segment of the ureter, and large inclusion-bearing cells were noted in the urothelium at the site of stenosis. In this respect the pathological findings in the ureter in cases 1 and 2 are almost identical with those described by Gardner and her colleagues in human polyomavirus infection in patient BK (Gardner et al., 1971; Coleman et al., 1973a).

Our observation in these three patients, cases 1 and 2 and BK, lead us to suggest that the administration of high-dose steroids may permit the activation of human polyomavirus and infection of ureteric epithelium, which has been damaged by ischaemia or inflammation. The excretion of polyomavirus after high-dose steroids may therefore be an indication to the clinician of ureteric damage where previously none had been suspected. Thus, by using cytological techniques in conjunction with virological, immunological, and histological studies where available, factors affecting activation of this virus in susceptible patients can be identified more precisely, and the natural history and degree of pathogenesis of this virus in immunosuppressed patients can be evaluated.

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Fig. 9 Case 2. Histological section of renal pelvis showing inclusion-bearing cells in the surface layers of the urothelium. H and E × 1250.
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Fig. 10 Case 2. Ultrathin section of ureter showing papovavirions in crystalline array in the nucleus of a urothelial cell. Uranyl acetate and lead citrate × 14000.

References


