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Invited Comment

BK-virus nephropathy in renal transplants—tubular necrosis, MHC-class II expression and rejection in a puzzling game

Volker Nickeleit[1], Hans H. Hirsch[2], Matthias Zeiler[1], Fred Gudat[1], Olivier Prince[1], Gilbert Thiel[3] and Michael J. Mihatsch[1]

[1]Institute for Pathology, [2]Institute for Medical Microbiology and [3]Department of Nephrology, Kantonsspital, University of Basel, Basel, Switzerland

Abstract We review BK-virus nephropathy (BKN) as a new complication that increasingly affects renal allografts and causes dysfunction. Since starting in 1996, we have seen 11 cases. Currently, the prevalence of BKN is 3% in our graft biopsies. The diagnosis can only be made histologically. The virus affects tubular epithelial cells that show characteristic intranuclear inclusion bodies. The major reason for impaired graft function and a possible way for viral particles to gain access to the blood via peritubular capillaries is necrosis of infected epithelial cells. BK-virus DNA in the plasma, which can be detected by PCR, is closely associated with nephropathy. BK-virus does not stimulate tubular MHC-class II expression as judged by immunofluorescence double labelling. The inflammatory response is inconsistent and the frequency of rejection episodes is not increased during disease. Clinical manifestation of viral nephropathy evolves in several stages. (i) Initial, asymptomatic and reversible activation of the virus, judged by the presence of inclusion bearing cells in the urine. (ii) High dose immunosuppressive drug regimens, often including tacrolimus. (iii) Tubular injury and viraemia as additional promoting conditions. BKN nephropathy was associated with graft loss in 45% of our patients. The remaining patients with persistent viral nephropathy showed renal dysfunction (serum creatinine levels on average 150% above baseline readings). Currently, no established antiviral therapy is available. We discuss attempts to lower immunosuppression as a means to control viral replication. We propose a diagnostic algorithm for screening and monitoring the disease.

Keywords: BK-virus; histology; ICAM; kidney; MHC-class II; outcome; polyomavirus; rejection; transplantation

Correspondence and offprint requests to: M. J. Mihatsch, MD, Institute for Pathology, Kantonsspital, Schoenbeinstr. 40, CH-4003 Basel, Switzerland.

Introduction

Manifest polyomavirus infection of renal allografts with the BK-virus strain, i.e. BK-virus nephropathy (BKN), is an unusual complication that has recently been described [1–3]. BKN causes severe graft dysfunction and contributes to graft loss [3–5].

Polyomavirus (PV), a subgroup of the papovavirus family, is a double stranded non-enveloped DNA virus [6]. Primary infection usually occurs early in life without clinical symptoms [6]. PV frequently remains in a dormant state in the kidneys and ureters of healthy, immunocompetent individuals [7–9]. Immunocompromised patients, however, have an increased risk of developing clinically manifest PV infection. Human disease can be caused by two PV strains: JC and BK. JC-virus causes progressive multifocal leukoencephalopathy [6,10]. BK-virus is associated with changes in the kidney [2,11] and, as proposed by some authors, with haemorrhagic cystitis and ureteral stenosis [12,13]. Although immunosuppression increases the probability of latent BK-virus reactivation, clinical manifestation of disease is rare.

In Basel we did not diagnose a single case of BKN before 1996, even during previous high-dose cyclosporin therapy protocols (personal observation). However, 10 patients with a total of 23 tissue samples were encountered in the following 3 years (current prevalence in transplant biopsies 3.1%, current prevalence in transplant recipients 4.5%). The prevalence in our centre is similar to that recently reported by Drachenberg *et al.* [4]. In comparison, a manifest CMV infection had been diagnosed in only 11 patients with a total of 16 graft biopsies over the past 32 years (prevalence in transplant biopsies 0.3%, prevalence in transplant recipients 0.9%). In the past 3 years (1996–1999) CMV was observed in one biopsy. Graft infections with adenovirus or herpes simplex virus have not been found in our centre. Thus, the recent onset and high prevalence of BKN points to new risk factors.

The following review is based on our personal experience with 11 well documented renal allograft

recipients suffering from BKN (one from an outside institution, see acknowledgement) as well as several cases seen in consultation. Special emphasis is placed on clinical and morphological aspects, risk factors, causes of graft dysfunction and the significance of interstitial inflammation.

BKN: morphological and clinical aspects

BKN is characterized by a histologically manifest renal allograft infection with the BK-virus strain and deteriorating graft function. Typically, viral DNA can be detected in the blood and inclusion bearing cells in the urine (see below). BKN is limited to kidney transplants and the attached ureters. There is no evidence that other organs, including the native kidneys, are affected in humans. In our experience, as well as that of others [4], neither ureteral obstruction nor haemorrhagic cystitis are features of BKN, although extragraft involvement has been described in a primate model [14]. We diagnosed BKN in 11 patients (eight men, three women; mean age at time of transplantation 52 years; range 27–61 years; seven cadaver kidneys and four living related donor kidneys). The initial diagnosis was made 9.6 months after transplantation (mean, range 2.8–25 months) in graft biopsies performed due to deterioration of renal function. Per definition, all graft biopsies ($n=23$) taken before the first diagnosis of BKN did not show viral changes (see below and Appendix A). Following the diagnosis of BKN, patients were observed on average for 373 days (mean, range 41–1105). 'Persistent BKN' is defined as the time interval during which tissue samples ($n=24$) showed histological evidence of ongoing viral infection (mean: 131 days; range: initial diagnosis 0–516 days).

Histological signs of BK-virus infection

The diagnosis of BKN can only be made histologically in a graft biopsy. The morphological hallmarks are intranuclear viral inclusions seen exclusively in epithelial cells, and focal necrosis of tubular cells. Four different variants of intranuclear inclusion bodies can be seen along the entire nephron (Figure 1). (i) Type 1 is an amorphous basophilic ground-glass variant: this type is most frequently found. (ii) Type 2, an eosinophilic, granular type surrounded by a (mostly incomplete) halo. (iii) Type 3, a finely granular form lacking a halo. (iv) Type 4, a vesicular variant with markedly enlarged nuclei and clumped, irregular chromatin. Of note type 2 inclusions bear similarities with changes seen in CMV infection. Type 4 inclusion bodies, which are primarily encountered in grafts with persistent BKN, are least characteristic and occasionally only distinguished after immunohistochemical incubations searching for PV (Figure 1 and Appendix B). Due to their clumped chromatin pattern and discernible nucleoli they resemble tumour cells. Cells with cytopathic changes are often (but not always) enlarged and can have pleomorphic nuclei, especially if they contain type 4 inclusions (Figure 1). Inclusion bearing cells can be particularly abundant in the medulla. A typical finding in BKN are infected cells that are rounded-up and extruded from the epithelial cell layer into tubular lumens (Figure 1). Viral replication often causes tubular epithelial cell necrosis with denudation of basement membranes (Figure 2). Thus, intratubular 'cellular' casts and denuded basement membranes can sometimes be a first diagnostic clue on low power microscopic examination drawing ones attention to an underlying viral infection. Although cytopathic signs are seen along the entire nephron, they are most abundant in distal tubular segments and collecting ducts. Only sporadically, infected cells are noted in the parietal epithelium lining Bowman's capsule, occasionally even forming small crescents [1]. In the renal pelvis and ureters, viral inclusion bodies (mostly type 1) can be seen in superficial (differentiated) transitional cells, rarely in the proliferating basal cell layer [1]. Podocytes, endothelial cells, mesenchymal cells and inflammatory cells appear not to be infected by BK-virus in human allografts. Changes in the interstitial compartment vary (see below). It is important to stress that these morphological changes are typical, but not pathognomonic for an infection with BK-virus. Herpes simplex virus, adenovirus and (less likely) even CMV must be considered in the differential diagnosis. They can easily be excluded by immunohistochemistry or electron microscopy [1] (see Appendix B). Ultrastructurally, PV presents with viral particles characteristically measuring between 30 and 50 nm in diameter that occasionally form crystalloid structures [1,2]. Immunohistochemistry and electron microscopy, however, serve only as ancillary techniques to confirm the diagnosis of BKN, since intranuclear inclusion bodies are always found by light microscopy [1,4]. In our patients a co-infection of the allografts with other viruses (CMV, EBV, herpes simplex types 1 and 2, adenovirus, Varizella) was not seen.

BKN and interstitial inflammation

Interstitial inflammation in BKN is poorly understood and the interpretation of the finding has remained controversial [1,3,4,14,15]. The major challenge is to distinguish between virally induced interstitial nephritis and cellular rejection. This distinction is not consistently made, yet seems to be crucial since the only current therapeutic option in BKN is reduction of immunosuppression [3,4]. Lowering the immunosuppressive regimen requires two pre-conditions: (i) absence of rejection and (ii) BK-virus should not trigger rejection.

In our experience, and that of others [4], BK-virus is accompanied by a heterogenous inflammatory reaction. In 17% of biopsies (4/24) taken during persistent BKN, inflammation was minimal to absent, particularly in the renal medulla (Figure 3, also see [4]). When inflammation was encountered the inflammatory

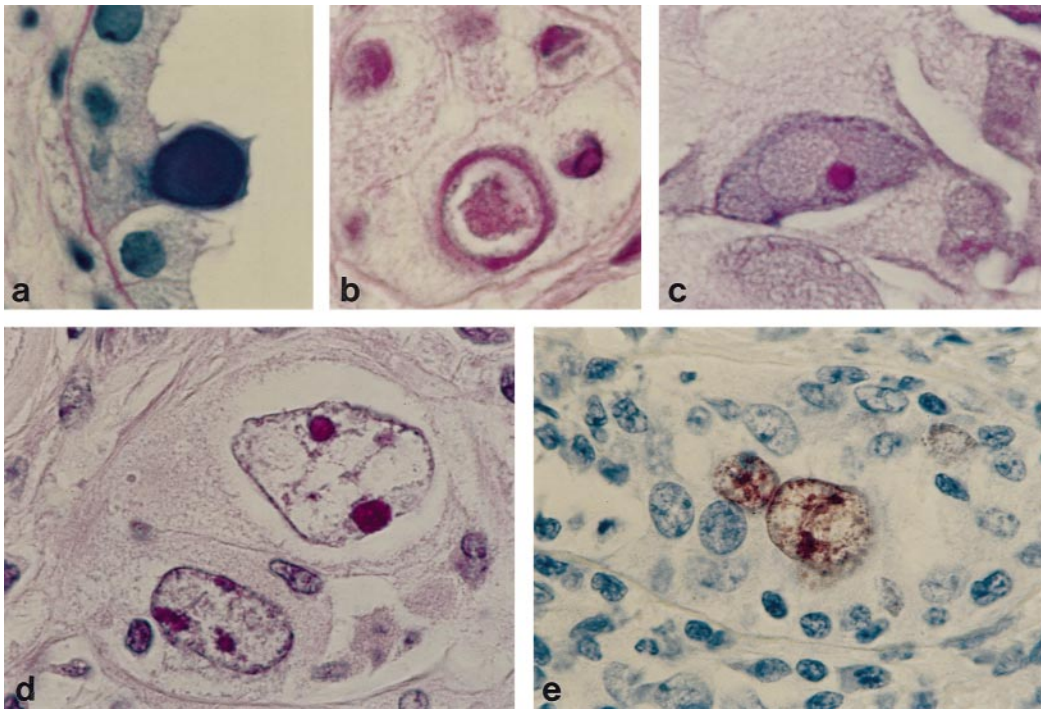

Fig. 1. Different types of intranuclear viral inclusion bodies in epithelial cells. (**a**) Amorphous ground-glass variant; PAS stain, 340 × original magnification. (**b**) Eosinophilic granular inclusion body surrounded by a halo; H&E stain, 340 × original magnification. (**c**) Centrally located finely granular type without a halo; H&E stain, 340 × original magnification. (**d**) Vesicular variant with irregular, clumped chromatin and nucleoli; H&E stain, 220 × original magnification. (**e**) Immunohistochemical detection of SV40 T antigen (red brown) in the nuclei of tubular cells with vesicular changes (same tissue specimen as pictured in d); 165 × original magnification.

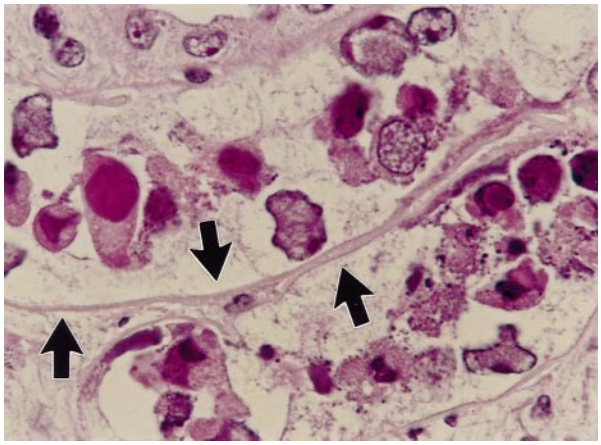

Fig. 2. Tubules with necrosis, and detachment of inclusion-bearing epithelial cells. Cellular debris and casts are found in the lumens; tubular basement membranes are segmentally denuded (arrows); H&E stain, 160 × original magnification.

cell infiltrate was composed of lymphocytes, macropahages and occasional plasma cells. Non-specific inflammation containing polymorphonuclear leukocytes was seen in response to markedly damaged tubules with urinary leakage (Figure 3, also see [4]). Tubulitis was frequently inconspicuous. Fifty-four per cent of biopsies performed during persistent BKN (13/24) showed evidence of cellular rejection as conventionally defined [16] with abundant tubulitis and transplant endarteritis in 23% (3/13). Typically, mononuclear cell infiltrates and tubulitis were pronounced in areas without viral inclusions making virally induced interstitial nephritis highly unlikely (Figures 3 and 4). Sporadically, infiltrates were rich in plasma cells; whether their presence, however, represents a specific virally induced inflammatory reaction is undetermined [14].

The upregulation of MHC-class II (HLA-DR) and ICAM-1 on tubular epithelial cells is a typical finding in graft biopsies with cellular rejection and can serve as an adjunct diagnostic tool [17,18]. HLA-DR expression can stimulate an allogeneic lymphocytic reaction and enhance T cell mediated lysis [19]. Thus, BK-virus could hypothetically trigger rejection episodes by inducing HLA-DR upregulation as previously proposed for CMV [20]. Virally stimulated HLA-DR expression would then render this marker useless for diagnosing rejection. However, our data do not support this hypothesis [21]. During persistent BKN the profile of tubular HLA-DR upregulation remained the same as observed in control tissue samples (Table 1). We did not find an association between BK-virus infection and tubular HLA-DR expression based on immunofluorescence double labelling staining techniques (Figure 3 and Appendix C). Inclusion bearing tubules were negative for HLA-DR and only inconsistently revealed weak ICAM-1 positivity. Even tubular cross sections with severe, virally induced injury and peritubular inflammation were negative for HLA-DR, however, expressed ICAM-1 (Figure 3). It was only in biopsies

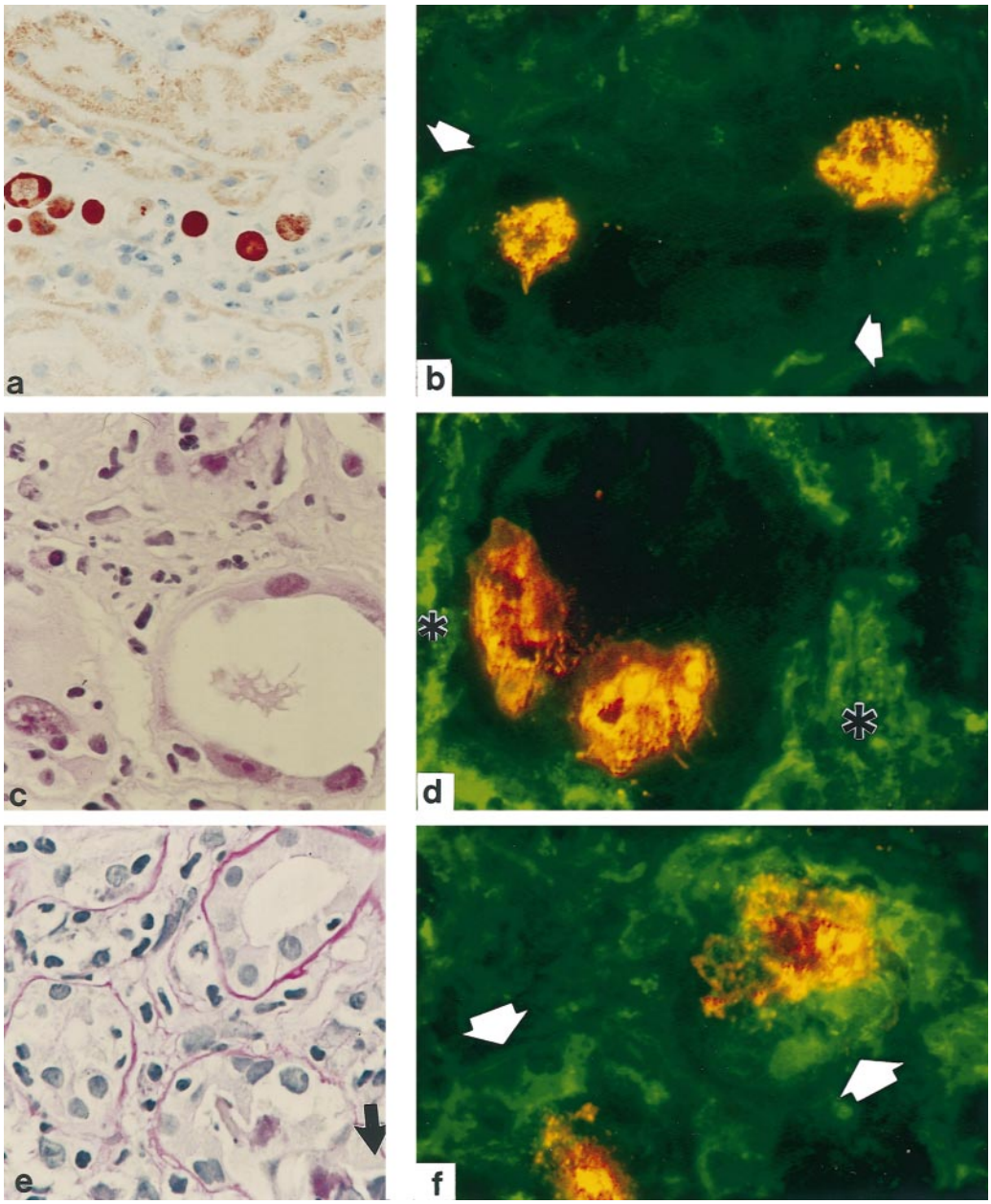

Fig. 3. Interstitial changes and tubular MHC-class II (HLA-DR) expression in BKN. (**a**) No inflammatory response is seen in the interstium adjacent to a tubule with inclusion bearing cells (red brown); immunohistochemical detection of SV40 T antigen, 110× original magnification. (**b**) Tubule with viral inclusion bodies (orange), HLA-DR is not detectable in tubular cells. The endothelium along peritubular capillaries normally expresses HLA-DR (arrows); immunofluorescence double labelling technique, polyomavirus in orange, HLA-DR in green, 110× original magnification. (**c**) A mixed inflammatory cell infiltrate (plasma cells, nuclear debris and polymorphonuclear leukocytes) is found adjacent to injured tubules showing dilatation and flattened epithelial cells; H&E stain, 110× original magnification. (**d**) Tubule with viral inclusion bodies (orange). HLA-DR is not detectable in tubular cells, however, is found in the adjacent interstitial inflammatory cell infiltrate (asterisks), same stain and magnification as (b). (**e**) Mononuclear cell infiltrates with widespread tubulitis and only few viral inclusion bodies in one tubule (arrow). This pattern is suggestive of rejection. (**f**) Area with tubulitis; tubules show viral inclusion bodies (orange) and upregulation of HLA-DR in tubular cells (green, arrows); 110× original magnification.

showing characteristic morphological evidence of rejection with marked tubulitis that we observed the typical tubular upregulation of HLA-DR and ICAM-1, regardless of the presence of inclusion bearing cells (Figure 3). Therefore, BK-virus did not stimulate HLA-DR expression. Rejection episodes were not induced either. We compared the prevalence of rejection in tissue samples taken during persistent BKN with time matched controls without BKN (Table 2). There was no significant difference found. Rejection was more common before BKN became manifest; particularly, rejection with transplant endarteritis ($P = 0.005$ in comparison to time matched controls).

Thus, BK-virus does not seem to provoke a constant

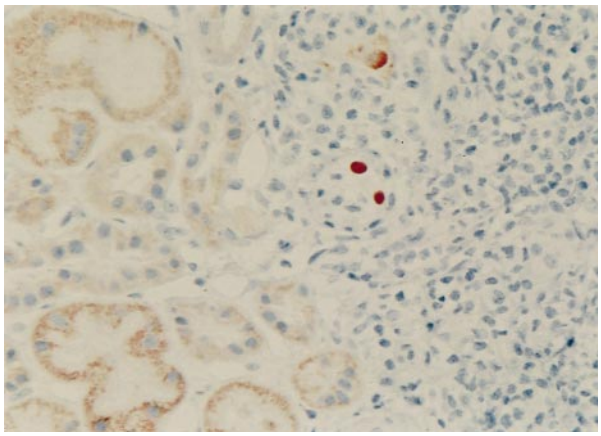

Fig. 4. Interstitial changes suggestive of rejection. Dense mononuclear cell infiltrates and tubulitis in an area with only scant viral inclusion bodies. Virally induced interstitial nephritis seems unlikely. Immunohistochemical detection of SV40 T antigen (red brown) in the nuclei of tubular cells; 80 × original magnification.

and pronounced interstitial inflammatory reaction. BKN is not associated with an increased prevalence of rejection episodes. Such a 'low inflammatory potential' could possibly be explained by a lack of virally induced tubular MHC-class II expression. However, rejection can coincide with BKN, an observation also made in a primate model [14]. In contrast to other authors

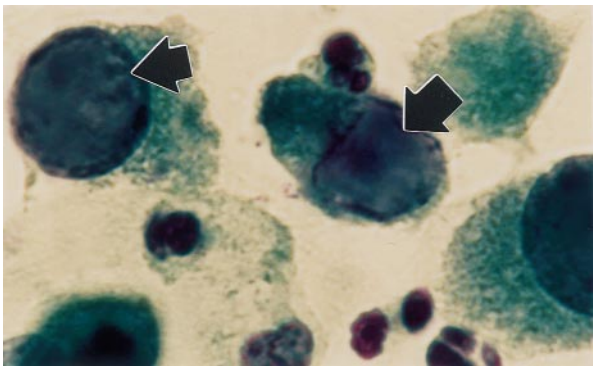

Fig. 5. Decoy cells in urine cytology. Amorphous ground-glass type intranuclear inclusion bodies are seen (arrows; compare with Fig. 1a). Papanicolaou stain, 400 × original magnification.

[3,15], we believe that in BKN the therapeutically crucial diagnosis of rejection can be made based on typical morphological changes accompanied by the characteristic tubular expression of HLA-DR.

Inclusion bearing 'decoy' cells in the urine

Inclusion bearing cells sloughed into the urine—presumably mainly of urothelial origin—can easily be detected in Papanicolaou stained cytological prepara-

Table 1. Profile of renal tubular MHC-class II (HLA-DR) expression in patients with and without BK-virus nephropathy. Listed are the numbers of positive biopsies/total number of biopsies evaluated (% positive)

Interstitial cellular rejection	Tubular MHC-class II expression Disease group		Control[a,b]
	before diagnosis of BK-virus nephropathy[b]	during persistent BK-virus nephropathy	
Yes (prominent tubulitis)	9/10 (90%)	8/8 (100%)	146/146 (100%)
No (minimal or no tubulitis)	3/9 (33%)	2/7 (29%)	107/272 (39%)

[a]Ref. [17].
[b]Biopsies did not show any evidence of BK-virus nephropathy.

Table 2. Prevalence of biopsy proven rejection episodes in patients with and without BK-virus nephropathy

Type of rejection	Before diagnosis of BK-virus nephropathy (time of biopsy post transpl. (median days))		During persistent BK-virus nephropathy (time of biopsy post transpl. (median days))		*P*-value[a]
	Disease group $n=23$[b] 29 days (range 8–207) positive biopsies (%)	Control group $n=69$[b] 27 days (range 12–209) positive biopsies (%)	Disease group $n=24$ 335 days (range 127–1105) positive biopsies (%)	Control group $n=72$[b] 338 days (range 212–741) positive biopsies (%)	Disease group before *vs* during BKN
Interstitial cellular rejection	12 (52%)	31 (45%)	13 (54%)	26 (36%)	n.s.
	n.s.		n.s.		
Transplant endarteritis	10 (44%)	9 (13%)	3 (13%)	8 (11%)	$P=0.02$
	$P=0.005$		n.s.		

[a]Fisher's exact test, *P* value; n.s. not significant.
[b]Biopsies did not show any evidence of BK-virus nephropathy.

tions (smears). Those cells were named 'decoy cells' [22,23] (Figure 5). They can be mistaken for tumour cells particularly if nuclei show vesicular changes (type 4 inclusions). Decoy cells are a characteristic but not a pathognomonic finding in patients with BKN (also see [4]). They were found in 11 of our patients in large numbers at the time of initial diagnosis (>5 decoy cells per 10 high power fields). In five out of six patients tested, decoy cell excretion preceded the histological diagnosis of BKN by a mean of 5.6 months (range: 1–12 months) when either renal function was unchanged or corresponding biopsies ($n=3$ available from two patients) showed no viral changes (see Appendix A). During persistent BKN decoy cells are a constant finding, however, they are sporadically low in number or even undetectable. Decoy cell excretion disappeared in patients overcoming BKN ($n=3$ evaluated, see below) as early as 2 days after graft nephrectomy in one patient with residual urine production. This suggests that decoy cells are likely of graft origin and do not originate from the native kidneys. Importantly, however, the detection of decoy cells in a renal allograft recipient does not necessarily mark BKN [1] (Figure 6). Their presence merely indicates an asymptomatic activation of PV that is generally fully reversible and not associated with renal dysfunction [22,23] (Figure 6). In a retrospective analysis of urine cytology reports, we found abundant decoy cell excretion (>5 decoy cells per 10 high power fields) in 7% (37/506) of sporadic urine samples from our Basel kidney transplant recipients. Generally, renal function was not impaired. The prevalence of decoy cells could possibly be even higher if urine is analysed carefully on a weekly or monthly basis (personal observation). BKN, on the other hand was only found in 27% of Basel patients with decoy cell excretion (10/37). Thus, the detection of abundant decoy cells as a diagnostic tool has a sensitivity of 100% but a positive predictive value of only 27%. The detection of BK-virus DNA in the urine by PCR is an inadequate tool for screening since the technique is by far too sensitive and for that reason even positive in patients without decoy cells.

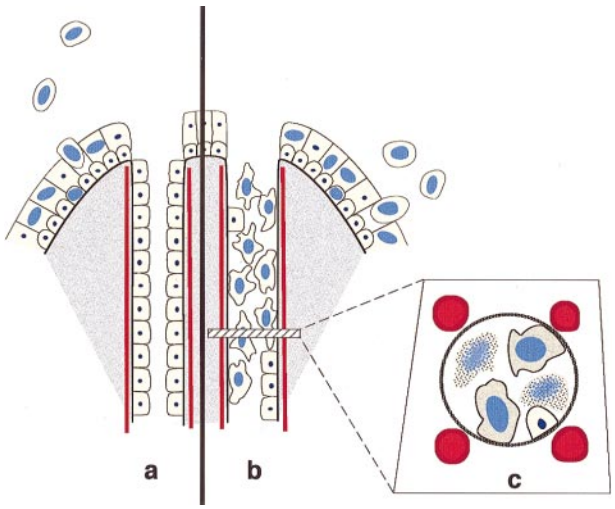

Fig. 6. Schematic of renal papillary tip and medulla. Intranuclear viral inclusion bodies are illustrated as large light blue 'nuclei'. (**a**) Reversible activation of polyomavirus without BKN. Tubules/collecting ducts are normal. Only the transitional cell layer shows inclusion bearing cells which are shed into the urine ('decoy cells'). (**b** and **c**) BKN, inclusion-bearing cells are present in renal tubules/ducts. Viral replication causes detachment and necrosis of tubular cells leading to denudation of basement membranes. This is a reason for graft dysfunction; compare with Fig. 2. (**c**) Cross section through duct/tubule. Lysis of tubular cells due to viral replication releases BK-virus into tubules with bare basement membranes. Viral particles can easily gain access to the blood by following the flow of fluid into the interstitium and to peritubular capillaries.

BK-viraemia in BKN

In the plasma of patients with BKN, viral DNA was constantly found by PCR in all patients (11/11; manuscript submitted for publication). The presence of viral DNA in the plasma was clinically asymptomatic, and not associated with fever or obvious viral spread to organs outside the kidney or urothelium. In four of eight patients evaluated, BK-virus DNA was detected in the plasma before the initial histological diagnosis of BKN (mean: 5.2 months, range: 2.6–8) when renal function was unchanged or corresponding biopsies ($n=3$ available from three patients) were without viral changes. Viral DNA disappeared from the plasma in five patients who overcame disease (see below). Following graft nephrectomy ($n=3$) BK-virus DNA was no longer detectable after a mean of 15 days (range 2–28), underlining the fact that viral DNA presumably originated from the renal graft. Only 5% of control transplant recipients without BKN (2/41) showed BK-virus DNA in the plasma. Thus, plasma PCR as a diagnostic tool has a sensitivity of 100% and a positive predictive value of 85%.

We speculate that viral DNA gains access to the blood stream once the activated virus does not only affect the urothelium, but also spreads to collecting ducts and tubules in the highly vascularized renal medulla (Figure 6). Presumably, BK-virus infects new cells via cell to cell spread as suggested by viral particles seen on apical tubular cell surfaces by electron microscopy [1]. Thus, BK-virus could follow an ascending route of infection from the superficial transitional cell layer to collecting ducts and tubules. Lysis of inclusion bearing tubular cells releases viral particles into the tubular lumen leaving behind denuded basement membranes. The virus may enter the blood stream when tubular fluid containing viral particles leaks into the interstitum that is rich in capillaries (Figures 6 and 7).

Risk factors promoting BKN

BKN seems to be promoted by the concurrent presence of several risk factors, among which immunosuppression is a prerequisite (Figure 8). It is conceivable, based on the early detection of decoy cells preceding BKN, that asymptomatic viral activation is an initial, fully reversible step in the development of nephropathy.

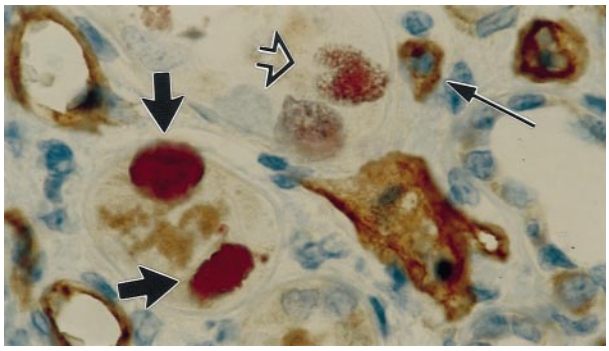

Fig. 7. Infected tubular cells with viral inclusions (red, arrows) are in close vicinity to peritubular capillaries (brown). One virally loaded nucleus seems to disintegrate (hollow arrow) right adjacent to a peritubular capillary (long arrow); compare with Fig. 6c. Immunohistochemical double labelling technique to detect SV40T antigen (red) and endothelium (CD34, brown), 160× original magnification.

The prevalence of decoy cells depends upon the intensity of immunosuppression. Most frequently i.e. in up to 30% of cases, they are found in patients receiving quadruple therapy (containing ATGam) and less often in patients under double therapy (0%, cyclosporin and prednison; personal observation). Another promoting factor, and possibly the most important one, may be 'high dose' administration of new immunosuppressive drugs such as tacrolimus [5]. In our series, 8/11 patients (73%) had been on a tacrolimus based regimen months before BKN became manifest. Trough levels of tacrolimus frequently exceeded 10–15 ng/ml ('rescue protocols'). The remaining 3/11 patients had been treated with mycophenolate-mofetil instead. In the recently published report by Randhawa *et al.* [3], 20/22 patients (90%) with BKN had also been treated with tacrolimus before manifestation of disease. In addition, it seems likely that specific changes in the allograft have to be present in order to promote BKN, since the native kidneys appear uninvolved. Tubular injury/regeneration could be such a condition rendering renal cells susceptible to BK-virus [24]. Ten out of 11 patients in our series suffered from rejection episodes in the months preceding BKN (biopsy proven in 9/10) and 2/11 had evidence of ischaemia (renal artery stenosis or cholesterol emboli). The spread of viral infection to the renal cortex could further be facilitated by viraemia. Once BK-virus gains access to the blood stream, it is possible that the virus 'homes' back to the cortex and infects tubular cells along the entire nephron.

Outcome

In our opinion, renal dysfunction in BKN is sufficiently explained by necrosis of tubular cells with denudation of basement membranes (Figure 2). Severe and long lasting tubular damage leads to consistent leakage of tubular fluid into the interstitial compartment which causes interstitial fibrosis and tubular atrophy, changes clearly linked to functional impairment [25]. Additionally, most of our patients suffered from rejection episodes that contributed to poor function and outcome.

We observed graft loss in 45% of patients (5/11; four nephrectomies, one patient on haemodialysis); on average 6 months post diagnosis (mean, range: 1–17 months). These graft failures were primarily seen in

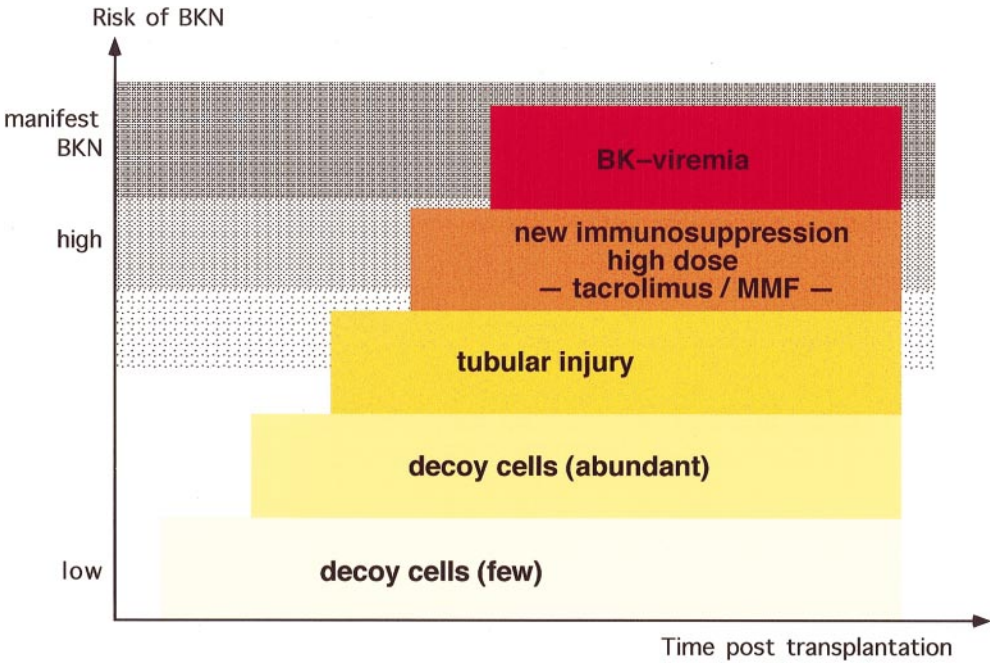

Fig. 8. Schematic of risk profile for the development of BKN. Note that several factors, which are closely linked to one another, have to concur to indicate high risk for disease. A key role seems to be played by new immunosuppressive drugs administered in high doses.

the first patients carrying the diagnosis of BKN in whom clinical experience was limited. Five of the eleven patients maintained graft function. They have been followed during persistent BKN for 12 months (mean, range: 3.5–27). Renal function deteriorated in all patients. On average, serum creatinine levels increased to >150% of baseline levels (mean ± SD, baseline: 153 ± 62 μmol/l; follow-up 237 ± 114 μmol/l).

Two out of 11 patients (one on haemodialysis mentioned above) cleared the virus after reduction of immunosuppression (biopsy proven clearance accompanied by disappearance of decoy cells from the urine and BK-virus DNA from the plasma). In one patient, clearance occurred within 2 months with return of the serum creatinine to baseline levels.

Patient management

Currently, no specific antiviral therapy is available to treat BKN. An attempt can be made to lower immunosuppression in an effort to give the organism a chance to clear the viral infection [3,4]. This approach is justifiable since BK-virus does not stimulate rejection. Our experience, however, is limited. Consequently, therapeutic recommendations can only be preliminary and are based solely on anecdotal personal observations.

The primary clinical goal is to early identify renal allograft recipients with BKN in order to attempt viral clearance, thereby limiting graft damage. Based on our experience, we propose the following diagnostic and therapeutic algorithm for patients under tacrolimus and/or mycophenolate mofetil immunosuppression. (i) Search for high numbers of 'decoy cells' in the urine (i.e. >5 decoy cells per 10 high power fields). (ii) If decoy cells are repeatedly present, perform PCR on plasma to search for BK-virus DNA. (iii) If plasma PCR is positive obtain a graft biopsy to establish a definitive diagnosis (including immunohistochemistry). (iv) If BKN is diagnosed, the immediate clinical response—in Basel—depends upon the presence or absence of concurrent rejection. In cases with rejection, we initially administer aggressive therapy that is followed by low dose immunosuppression, preferably cyclosporin based. Of note, not until immunosuppresion is lowered and virally induced tubular necrosis starts to disappear will renal function improve significantly. (v) In order to monitor the treatment effect of low-dose immunosuppression i.e. viral clearance, we perform PCR on plasma samples and quantify decoy cells in the urine. PCR analysis on plasma samples appears particularly useful as a non-invasive tool to screen for viral clearance and to limit the duration of low-dose immunosuppression. During low dose immunosuppression, one should have a high level of suspicion of rejection episodes.

There is evidence that this approach can be beneficial. We recently identified a patient with BKN based on urine and plasma screening. Two patients cleared the virus after reduction of immunosuppression, one of whom had transiently been treated aggressively with bolus steroids following the diagnosis of BKN and concurrent rejection. This patient subsequently returned to baseline renal function. So far we have not observed 'explosive' viral spread in association with pulse steroid therapy. The proposed algorithm will hopefully permit a better understanding of BKN, its specific risk factors and its effective treatment strategies.

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Appendix

A. Biopsies obtained before the diagnosis of BKN

Nine patients' graft biopsies ($n=23$) were obtained during the time interval before BKN was first diagnosed. These biopsies were free of viral inclusions judged by light microscopy, electronmicroscopy and immunohistochemistry

B. Immunohistochemical detection of polyomavirus

Immunohistochemically, polyomavirus (SV40 large T antigen, which is common to JC-virus, BK-virus and the simian virus 40) can easily be detected with the indirect peroxidase technique on formalin fixed, paraffin embedded samples as well as frozen tissue and cytological preparations. We used a mouse monoclonal antibody (mAb) (Calbiochem/Oncogene Research Products, Cambridge, MA, USA, Cat # DP02, clone PAb 416 overnight incubation at 4°C, dilution, 1 : 2000) combined with the microwave technique for antigen retrieval (Tris–HCl buffer 0.5M, pH 10.5, 15 min at 95°C) and the avidin–biotin technique as indicator system

C. Immunofluorescence double labelling to detect polyomavirus and either MHC-class II or ICAM-1

Polyomavirus and MHC-class II. mAb detecting the SV 40 large T antigen (see above) followed by a Texas Red conjugated secondary antibody. MHC-class II expression was evaluated by direct IF using a FITC conjugated mouse anti human mAb (DAKO A/S, Glostrup, Denmark; dilution 1 : 4; 30 minutes incubation at room temperature)

Polyomavirus and ICAM-1. FITC conjugated mouse mAb detecting the SV-40 large T/small t antigen (PharMingen, San Diego, CA, USA; dilution 1 : 10, overnight incubation at 4°C); ICAM-1 expression was evaluated with a mouse mAb (Immunotech, Marseille, France; dilution 1 : 25, 1 h at RT) followed by a Texas Red conjugated secondary antibody