Nephrol Dial Transplant (2007) 22: 2386–2390
doi:10.1093/ndt/gfm283
Advance Access publication 2 June 2007

NDT
Nephrology Dialysis Transplantation

Case Report

Transient allograft dysfunction from immune reconstitution in a patient with polyoma BK-virus-associated nephropathy

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Keywords: immune reconstitution syndrome; polyoma BK-virus nephropathy; renal allograft rejection

Introduction

During the last 10 years, polyoma BK-virus associated nephropathy (PVAN) has emerged as a serious complication in renal transplant recipients [1]. Due to the establishment of an accurate non-invasive screening procedure measuring polyomavirus BK-viraemia, BK-viruria and decoy cells in urine, PVAN can be diagnosed at early stages [2]. This allows for timely therapeutic intervention, which has significantly reduced the incidence of severe PVAN courses including graft loss [3,4].

Management of PVAN is mainly based on a reduction of the immunosuppressive drugs, while the impact of anti-viral therapy is not yet clear [5]. This strategy bears the inherent risk that allograft rejection may arise, which is difficult to differentiate from an immune response to the BK-virus, because both entities can present as morphologically and molecularly indistinguishable, with interstitial infiltrates and tubulitis [1,5–7]. Therefore, more data regarding the natural course of PVAN under reduced immunosuppression might be helpful to illuminate the scope of post-intervention responses.

Case report

A 37-year-old woman had end-stage renal failure due to a nephropathy of unknown origin. She was highly sensitized as a consequence of two blood transfusions and two pregnancies (peak CDC-PRA 78%, peak FlowPRA^{TM} class I 93%, FlowPRA^{TM} class II negative). After being on haemodialysis for 9 years, she received a kidney from a 7-year-old deceased donor. There were three HLA-mismatches (recipient: HLA-A3/24, B7/55, DR4/13; donor: HLA-A2/24, B7/38, DR11/13) and the recipient had two donor-specific HLA-antibodies (DSA) detectable in three historic sera (A2 and B38; determined by FlowPRA^{TM} single-antigen flow-beads). Flow-cytometric T- and B-cell cross-matches were positive with historic sera, but negative with the current one. The patient was considered to be at high risk for rejection and received an induction therapy consisting of polyclonal anti-T-lymphocyte globulin (ATG-Fresenius) as well as intravenous immunoglobulins (IvIg) [8]. Maintenance immunosuppression consisted of tacrolimus, mycophenolate mofetil (MMF) and steroids.

Figure 1 summarizes the course of immunosuppressive therapy, allograft function and BK-virus activity; Figure 2 demonstrates the histology of the four allograft biopsies obtained in this patient. The allograft had an immediate good function and serum creatinine dropped to 140 μmol/l by day 10 post-transplant. On day 21, serum creatinine rose to 308 μmol/l and the first allograft biopsy was obtained. The diagnosis of antibody-mediated rejection was made based on the presence of thrombotic microangiopathy, diffuse C4d-staining in peritubular capillaries and reappearance of both remote DSA (A2 and B38) in high quantities (Figure 2; picture 1A and 1B). The patient received another course of IvIg, six steroid pulses and four plasmapheresis treatments. Subsequently, serum creatinine declined to 110 μmol/l.

Eight weeks post-transplant, increasing BK-virus replication in the urine was noted along with the appearance of numerous decoy cells, followed by plasma BK-viral loads persisting above 10 000

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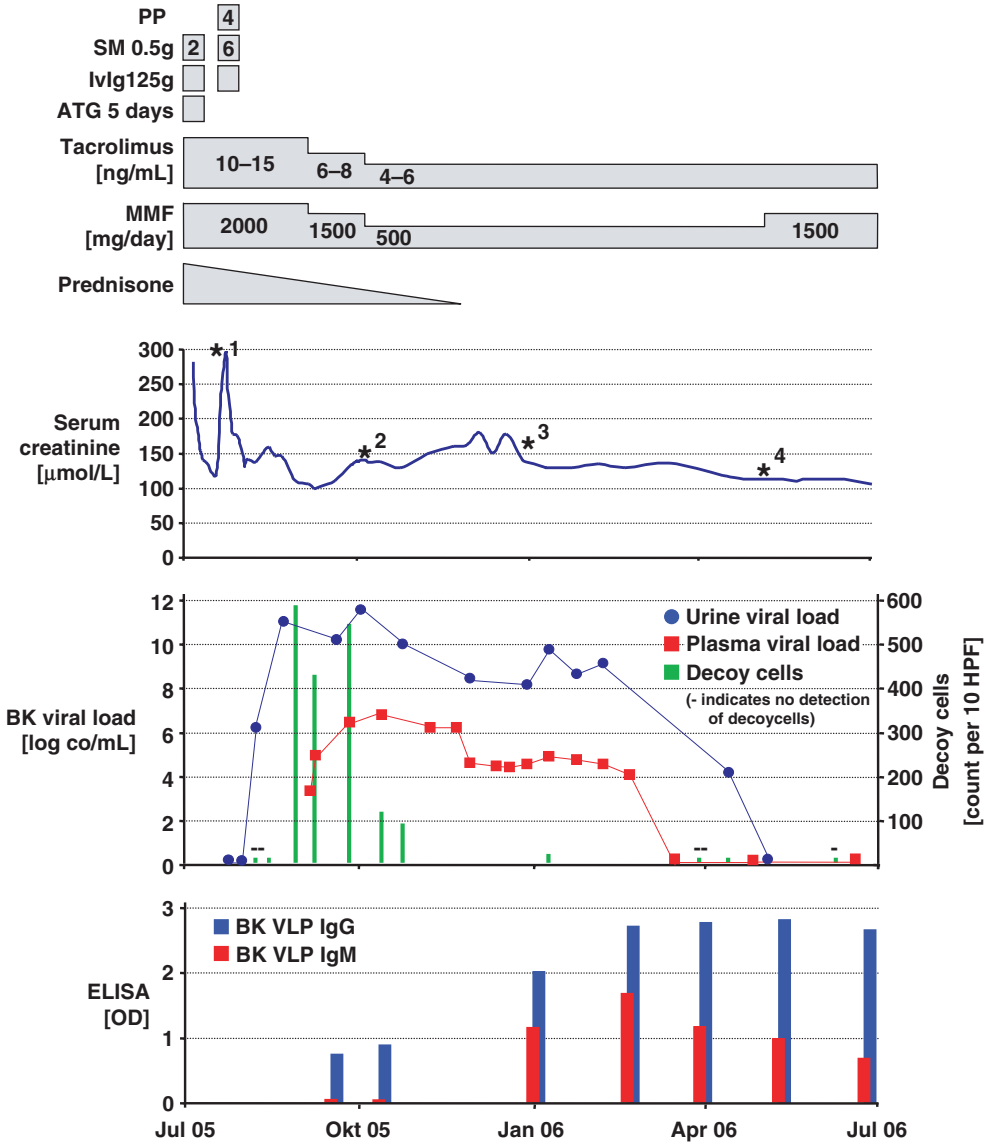

Fig. 1. Course of immunosuppressive therapy, allograft function, BK-virus activity and BK-virus serology within the first year after transplantation. Stars with appended numbers in the serum creatinine graphic indicate allograft biopsies. The corresponding allograft histologies are shown in Figure 2. PP, plasmapheresis; SM, solumedrol i.v.; IvIg, intravenous immunoglobulins; BK-virus-specific IgG and IgM antibodies were measured in human sera using virus-like particles (VLP) as antigens coated to microtitre plates. BK-virus-like particles were purified by density gradient centrifugation of lysates from Sf9 insect cells infected with recombinant BK-virus-VP1 Bac-to-Bac expression vectors (Invitrogen). Antigen coating and serum dilutions provided optimal OD results at 50 ng and 1: 400, respectively (S. Bodaghi and H.H. Hirsch, unpublished data).

copies/ml. The diagnosis of 'presumptive' PVAN was made [1] and immunosuppression reduced (tacrolimus trough levels from 10–15 ng/ml to 6–8 ng/ml, MMF dose from 2 to 1.5 g/day, further tapering of steroids). Three weeks later, a second allograft biopsy was performed. Dense focal lymphohistiocytic infiltrates were seen, affecting 20% of the cortical and 35% of the medullar area with mild tubulitis. There were numerous SV40 antigen-positive tubular epithelial cells in the medulla and in the cortex (Figure 2; picture 2A and 2B). At this time-point, BK-viral loads were 3.9×10^{11} copies/ml in urine and 5.1×10^{6} copies/ml in plasma, respectively. The diagnosis of PVAN pattern B was made and immunosuppression further reduced (tacrolimus trough levels to 4–6 ng/ml, MMF dose to 0.5 g/day, steroids were tapered out over 8 weeks).

BK-viraemia remained constantly above 10^6 copies/ml for 8 weeks, but then started to decline by two log10 units to a plateau of around 4×10^4 copies/ml. Within this time period, serum creatinine concentrations slowly rose from 140 to 180 μmol/l. A third allograft biopsy was performed, which showed massive diffuse lymphohistiocytic infiltrates affecting 100% of the cortical and medullar area with severe tubulitis. There were only few SV40 antigen positive tubular epithelial cells in the medulla and the cortex (Figure 2; picture 3A and 3B). A presumed diagnosis of BK-virus specific immune reconstitution was made.

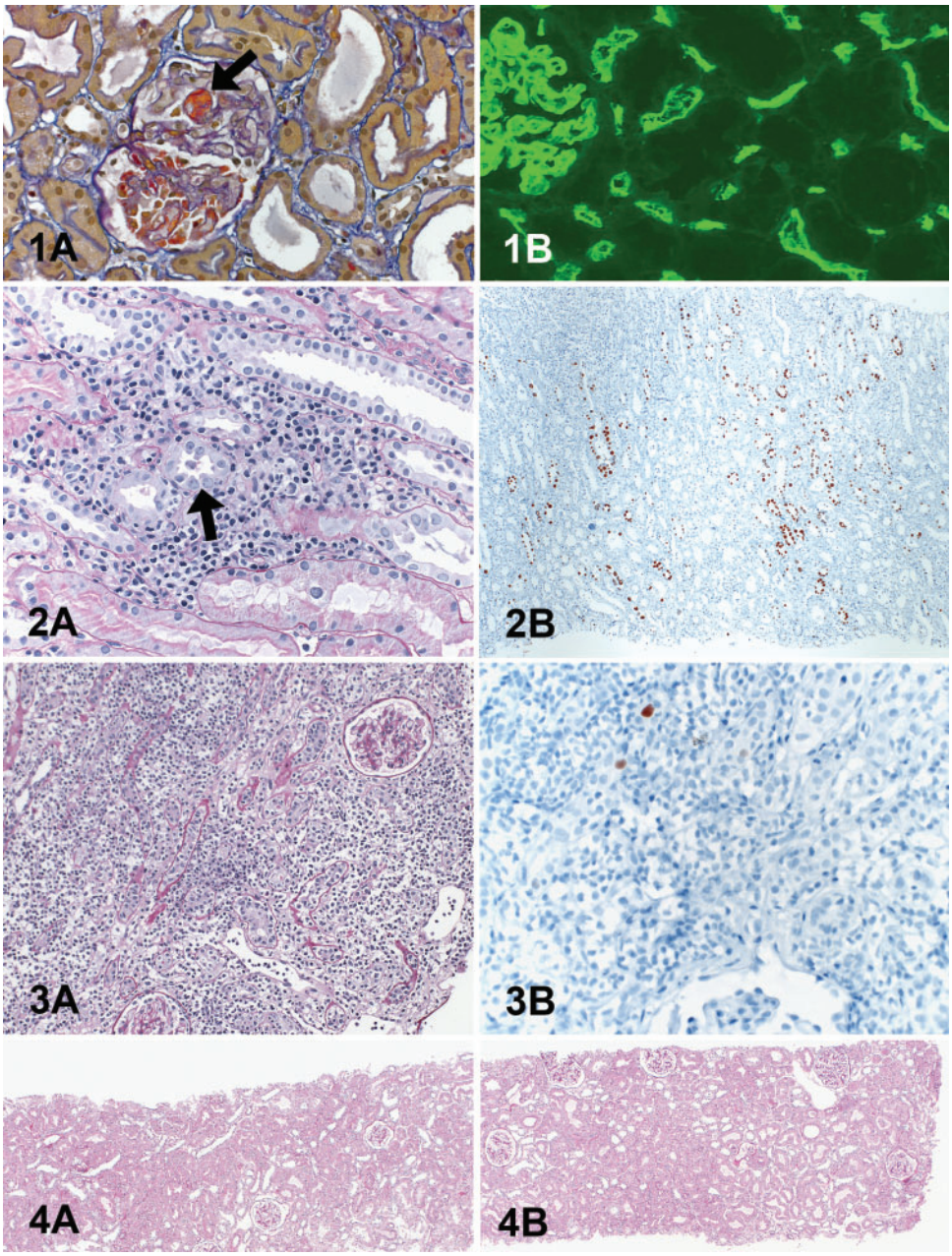

Fig. 2. Allograft histology graded according to the Banff classification [16] and a recently proposed semi-quantitative assessment for PVAN [1]. All allograft biopsies consisted of two cores obtained with a 16-gauge needle. **First biopsy**, 21 days post-transplant (cy0, i0t0, ci0ct0): the tubulointerstitial compartment was completely normal, but there were fibrin thrombi in peripheral glomerular capillary loops (arrow) consistent with thrombotic microangiopathy (**1A**). Peritubular capillaries (PTC) were diffuse positive for C4d by immunofluorescence (IF) (**1B**). Staining for SV40 antigen by immunohistochemistry was negative. **Second biopsy**, 3 months post-transplant (cy3, i2t1, ci0ct0): dense focal lymphohistiocytic infiltrates in the cortical and medullary interstitial space with mild tubulitis. Note enlarged nuclei (arrow) without clear-cut inclusion bodies (**2A**). Glomeruli and arteries were completely normal. Staining for SV40 antigen revealed numerous infected tubular epithelial cells in the cortex and medulla (**2B**). Staining for C4d in PTC and HLA-DR in tubular epithelial cells were negative. **Third biopsy**, 6 months post-transplant (cy1, i3t3, ci0ct1): massive diffuse lymphohistiocytic infiltrates affecting the whole cortical and medullary space with severe tubulitis (**3A**). Immune phenotyping of the infiltrate revealed a very dominant fraction of T-cells (CD3+) with equal amounts of CD4+ and CD8+ cells, few macrophages (CD68) and few B-cells (CD20). Glomeruli and arteries were completely normal. Only few tubular epithelial cells were positive for SV40 antigen (**3B**). Staining for C4d in PTC and HLA-DR in tubular epithelial cells were negative. **Fourth biopsy**, 10 months post-transplant (cy0, i0t0, ci0ct0): both biopsy cores showed perfectly normal renal tissue (**4A** and **4B**). No tubular epithelial cells were positive for SV40 antigen. Staining for C4d in PTC and HLA-DR in tubular epithelial cells were negative.

Immunosuppression consisting of tacrolimus with trough levels of 4–6 ng/ml and MMF 0.5 g/day was maintained and no additional therapy introduced. Retrospective analysis of serum samples at this time-point revealed newly produced BK-virus specific IgM together with increasing IgG antibodies (Figure 1, bottom panel) consistent with an emerging BK-virus specific immune response.

Ten weeks later, BK-viraemia became negative, followed by clearance of the virus in the urine. Serum creatinine declined and stabilized at 110–120 μmol/l. A follow-up biopsy showed normal allograft tissue without any tubulointerstitial infiltrates, no tubular atrophy and interstitial fibrosis and no SV40 antigen positive tubular epithelial cells (Figure 2; picture 4A and 4B). Testing for BK-virus-specific T-cells by interferon-γ ELISpot at this time-point revealed a response against BK-virus large T and VP1 proteins. Intracellular staining for interferon-γ showed a prominent large T- directed CD8+ T-cell response consistent with an established cellular immune response as reported previously (Figure 3) [9].

Discussion

The majority of patients with PVAN demonstrate tubulointerstitial inflammation [6,10], which can either indicate concurrent allograft rejection or an inflammatory response to the BK-virus. The relative contribution of these two entities is unknown and specific single markers to separate them are currently lacking. The clinico-pathological course of our patient is intriguing and may serve as an index case of BK-virus-specific immune reconstitution syndrome.

At the time of the third allograft biopsy showing diffuse massive interstitial infiltrates and severe tubulitis, circumstantial evidence suggested that acute rejection was less likely as the cause. First, extensive infiltrates were coincident with a significant decline of BK-virus replication, indicated by a decreasing plasma BK-viral load of >2 log10 units and a substantial clearance from the allograft based on a significant reduction of SV40 antigen-positive tubular epithelial cells. Second, the increase of serum creatinine was only moderate, despite severe tubulointerstital pathology. Third, staining of tubular epithelial cells for HLA-DR in frozen sections was available, which served as an adjunctive tool to differentiate between tubulitis due to rejection and PVAN. HLA-DR positivity is a typical finding in Banff Ia/Ib rejection whereas it is mostly negative in PVAN, as in this case and described earlier [11]. However, the strongest argument against an alloimmune-related inflammation was the clinico-pathological course. Indeed, massive tubulointerstitial infiltrates developed after immunosuppression was significantly lowered, but they subsequently resolved completely while maintaining the same low immunosuppression without having applied any rejection treatment. A drug-induced interstitial nephritis is also very unlikely, because no drugs were added or discontinued within this time frame, with the exception of tapering out prednisone and reducing tacrolimus and MMF. Therefore, BK-virus-specific immune reconstitution is the most probable explanation for the observed clinico-pathological course, and to the best of our knowledge there is no consistent alternative diagnosis.

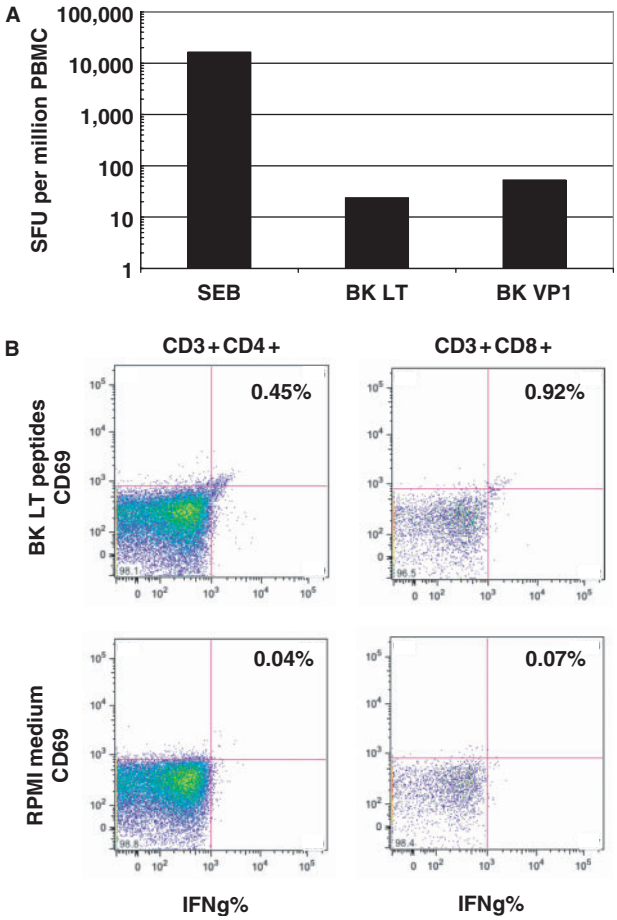

Fig. 3. BK-virus-specific interferon-γ production in PBMC at resolution of PVAN (i.e. at the time point of the fourth allograft biopsy). The 15-mer peptide pools of 11 amino acid overlaps spanning BK-virus large T-antigen (LT) and VP1 capsid protein (VP1) were used for PBMC stimulation and interferon-γ detection by ELISpot assay (**A**) or by intracellular cytokine staining/flow cytometry (**B**) as described previously [9]. *Staphylococcus enterotoxin B* (SEB, SIGMA, Buchs, Switzerland, 1 μg/ml) and cell culture medium served as positive and negative control, respectively. The number of spot forming unit (SFU) per well was calculated from triplicates after subtractions of negative control. By flow cytometry, at least 30 000 CD3+ cells were acquired and analysed on a FACS-Canto (Becton Dickinson). The frequency of BK-virus-specific cellular immune responses was determined for each antigen and expressed as percent of interferon-γ positive cells among CD3+CD4+CD69+ or CD3+CD8+CD69+ gated lymphocytes, respectively.

Support for this interpretation comes from retrospectively measured BK-virus-specific antibody titres as a surrogate marker of the emerging immune response to the BK-virus, as has been reported previously [12]. A marked increase of BK-virus specific IgM and IgG was noted following the decline of plasma BK-virus load at the time of severe tubulointerstitial inflammation. Longitudinal analysis of BK-virus specific cellular immune responses or characterization of cellular infiltrates in the allograft would have been of particular interest in this case [13], but were not available. However, we analysed the frequency of BK-virus specific interferon-γ producing

T-cells by ELISpot and flow cytometry after resolution of PVAN, which were consistent with an established cellular immune response against the BK-virus and in line with our previous observation [9]. The reported case suggests that integration of longitudinal dynamics of BK-virus replication together with allograft function, histology and possibly markers of virus-specific cellular and humoral immunity may currently be the most valuable parameter for differentiation of tubulointerstitial allograft rejection *vs* a BK-virus-specific immune response.

An interesting finding in this case was the detection of completely normal renal tissue in the last biopsy despite severe tubulointerstitial inflammation and many BK-virus infected tubular epithelial cells in previous biopsies. This finding is surprising and may indicate that the end-stage of PVAN characterized by progressive tubular atrophy and interstitial fibrosis is the result of ongoing tubular cell necrosis unbalanced by regeneration [10]. Therefore, the good outcome in the reported case could be related to an early and efficient clearance of the BK-virus, or to a high capacity of the allograft from a 7-year-old donor to sustain injury and to regenerate. Although this single case suggests that even severe inflammation due to immune reconstitution may not be harmful, the value of immunomodulatory drugs including steroids and leflunomide as a treatment option to limit the extent of inflammation is not clear yet. Notably, the immune reconstitution syndrome is well-known in HIV/AIDS patients and steroids are often considered when the inflammation becomes damaging to an organ [14,15].

In conclusion, we report a case consistent with BK-virus-specific immune reconstitution after reduction of immunosuppression for treating PVAN, leading to transient severe tubulointerstitial inflammation and moderate allograft dysfunction. Clearly, no general recommendations regarding diagnosis and treatment of BK-virus specific immune reconstitution syndrome can be made based on this single case. Prospective studies with standardized management are required to validate our findings and to further elucidate aetiology and treatment of tubulointerstitial infiltrates in patients with PVAN.

Acknowledgement. S.S. is supported by Swiss National Foundation Grant 3200B0-109302. H.H.H. is supported by Swiss National Foundation Grant 3200B0-110040/1. and by Stiftung Forschung Infektionskrankheiten, Basel (SFI#6-2005).

Conflict of interest statement. None declared.

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Received for publication: 11.4.07
Accepted in revised form: 16.4.07