PATHOPHYSIOLOGY of the RENAL BIOPSY

Adenovirus Interstitial Nephritis and Rejection in an Allograft

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ABSTRACT
Viral infections are an important complication of solid organ transplantation. Although polyoma is the virus that most commonly infects the renal allograft, adenoviral infections are also reported. We describe the clinical and pathologic findings in a patient with adenoviral infection associated with acute rejection of the renal allograft. The pathologic findings of adenovirus infection usually include a granulomatous interstitial nephritis, which is helpful in distinguishing from acute rejection. We discuss the differential diagnosis and pathophysiology of allograft viral infections and concomitant rejection.


Although the advent of intensified immunosuppressive protocols has led to lower rates of acute rejection, opportunistic viral infections are increasingly occurring.1 Such infections of the allograft can present significant challenges in both their diagnosis and management.

CASE PRESENTATION
A 54-year-old male with end-stage renal disease due to hereditary nephritis received a kidney transplant from a living unrelated donor. He received standard immunosuppression with tacrolimus, mycophenolate, and prednisone; no induction therapy was given. His serum creatinine was 156 μmol/L (1.8 mg/dl) 1 week after surgery. He was also started on valganciclovir within the first week for esophagitis, possibly viral, although tissue viral cultures and biopsy stains for herpes simplex virus (HSV) and cytomegalovirus (CMV) were ultimately negative.

He was reported to have decy cells in urine cytology on routine monitoring 12-days posttransplant, and this finding persisted. Weekly serum PCR for BK and JC viruses were negative throughout the posttransplant course. At 6-weeks posttransplant, his creatinine rose to 198 μmol/L (2.1 mg/dl), and an allograft biopsy was performed.

Allograft Biopsy 1
A severe granulomatous interstitial nephritis was observed, with palisading tubulo-centric granulomas, severe tubular epithelial injury with regeneration, and viral cytopathic changes, with enlarged, hyperchromatic, smudgy nuclear inclusions (Figures 1, 2, and 3). Severe lymphocytic tubulitis, diffuse severe peritubular capillaritis with mononuclear cells and marginating neutrophils, and a focus of small vessel endothelialitis without fibrinoid injury (Figure 4) were also present, consistent with acute rejection (Banff grade IIA, Banff scores: g0,i3,t3,v1,ptc3,cg0,mm0,c10,ct0,cv0,ab0, C4d negative). Stains for acid-fast bacilli and fungal organisms were negative. The interstitial infiltrate was a mixture of CD3+ T cells, CD79a+ B cells, and CD68+ macrophages, with some scattered CD20+ B cell aggregates. Immunocytochemical stains for SV40, CMV, HSV, and adenovirus (ADV) were all negative, as was in situ hybridization for BKV, JCV, and EBV. In situ hybridization for ADV DNA (Dr. P. Randhawa, University of Pittsburgh Medical Center, Pittsburgh, PA) was positive in a single cortical tubule (Figure 5). No sample was taken for electron microscopy studies.

Urine for viral culture grew ADV. Serum for donor-specific antibody was negative. He was initially treated with intravenous immunoglobulin (IVIG) and pulse steroids; the mycophenolate was subsequently withheld when ADV infection was confirmed. One week later, the serum creatinine had risen to 283 μmol/L (3.2 mg/dl), and a repeat allograft biopsy was performed.

Allograft Biopsy 2
There was ongoing acute tubulointerstitial rejection (Banff grade IB) with moderate acute transplant glomerulitis (mononuclear cells and neutrophils), severe interstitial inflammation, severe lymphocytic tubulitis with focal tubular basement membrane disruption, and diffuse severe peritubular capillaritis with mononuclear cells and occasional...
neutrophils (Banff scores: g2,i3,t3,v0,ptc3, cg0,mm0,ci0,ct0,cv1,ah1, C4d negative). There was a focus of arteriolitis with marginating mononuclear cells, but no evidence of intimal arteritis. On this biopsy, there were no definitive viral cytopathic changes and no granulomatous interstitial inflammation. The interstitial infiltrate was predominantly CD3+ T cells, with a population of CD68+ macrophages and a minority of CD20+ B cells. Cells infiltrating glomeruli included CD68+ and CD3+ cells. No specific stains for ADV were performed on this biopsy.

The patient received a second pulse of oral steroids and mycophenolate was restarted. His serum creatinine slowly improved to the 140 to 160 μmol/L range. Urine culture for ADV remained positive until 3 months posttransplant, when it became negative; urine cytology continued to show viral cytopathic changes for some months thereafter.

Allograft Biopsy 4
A protocol allograft biopsy at 6-months posttransplant showed mild tubulointerstitial inflammation (Banff borderline), mild patchy tubular atrophy and interstitial fibrosis (affecting approximately 10% of cortex), and moderate intimal fibrosis (Banff scores: g0,i1,t1,v0,ptc0, cg0,mm0,ci1,ct1,cv2,ah0, C4d negative). The patient is now 24-months posttransplant, with a stable serum creatinine of 130 μmol/L (1.5 mg/dl).

DISCUSSION
Adenovirus Infection of the Renal Allograft
ADV infection in kidney transplant recipients is relatively common, with viremia present in 6.5% of renal transplant recipients within the first year. ADV infection of the allograft itself appears to be much less common, although numerous case reports exist. Patients usually present with hemorrhagic cystitis and hematuria, fever, or both.

Methods for diagnosing ADV infection include urine culture for ADV, urine for electron microscopy, or serum PCR to establish viremia. Renal biopsy is required for definitive diagnosis. Viral cytopathic changes (decoy cells), which are typically associated with polyomavirus infection, have been previously reported in the urine of transplant recipients with ADV infection. Our patient demonstrated decoy cells in his urine over the course of a few months, during which time his urine culture was positive for ADV and serum PCR for BKV virus was negative. Urine culture and urine PCR for BKV and urine electron microscopy are not done routinely at our center; thus, we cannot definitively rule out the possibility of a concomitant BKV infection contributing to the decoy cells.

The clinicopathologic features of renal allograft viral infections are summarized in Table 1. The main differential diagnosis is polyomavirus infection, as CMV infection in the allograft is exceptionally rare. The tubular viral cytopathic features of ADV replication, with hyperchromatic ground glass or smudgy in-
tranuclear inclusions (Cowdry A), are similar to the type I inclusions of BKV. They are usually more pronounced in ADV, however, with associated severe tubular epithelial necrosis, inflammatory infiltration, tubular basement membrane disruption, and frank tubular destruction.

Severe necrotizing granulomatous tubulointerstitial nephritis with palisading tubulo-centric granulomas surrounding severely injured and virally infected tubules appears to be characteristic of ADV infection.\(^5,6\) The distal nephron is primarily affected, with interstitial nephritis maximal in the medulla and at the cortex-medullary junction. Granulomatous interstitial nephritis, with non-necrotizing epithelioid granulomas surrounding injured tubules, has only rarely been reported in BKV-associated allograft nephropathy.\(^7\) Macrophage recruitment appears to be prominent in the biology of renal ADV infection, and indeed in viral infections in general. Why granulomatous interstitial nephritis is more prominent in ADV infections compared with other renal viral infections is not clear, but may reflect the underlying cytokine reaction of renal tubular cells infected by ADV. Other potential causes of granulomatous interstitial nephritis must be excluded, including drugs, sarcoidosis, anti-neutrophil cytoplasmic autoantibodies (ANCA)-associated vasculitis, tuberculosis, and fungal infections.

Viral Infection and Rejection in the Allograft
The relationship between infection and allograft rejection remains controversial,\(^8\) and it is probable that these two processes may be related either directly or indirectly.

### Indirect Relationship

There are two considerations in an indirect relationship. First is increased immunosuppression facilitating infection. In some circumstances, acute rejection may be the initial event, and the subsequent infection.

### Table 1. Clinical and pathologic features of renal allograft viral infections

<table>
<thead>
<tr>
<th></th>
<th>Adenovirus</th>
<th>Polyomavirus</th>
<th>Cytomegalovirus</th>
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<tbody>
<tr>
<td><strong>Prevalence</strong></td>
<td>Approximately 7%</td>
<td>Approximately 12%</td>
<td>Highly variable</td>
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<tr>
<td><strong>Viremia</strong></td>
<td>Rare, &lt;0.5%</td>
<td>Rare</td>
<td>Extremely rare</td>
</tr>
<tr>
<td><strong>Graft infection</strong></td>
<td></td>
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<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic symptoms</td>
<td>Common</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ involvement</td>
<td>Lung, GI tract, disseminated</td>
<td>Kidney</td>
<td>Lung, liver, GI tract, disseminated</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Potentially life threatening, but reversible</td>
<td>Risk of persisting infection leading to graft failure</td>
<td>Not a recognized cause of chronic graft failure</td>
</tr>
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<td><strong>Viral inclusion types</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nuclear</td>
<td>++</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Ground glass, homogenous</td>
<td>±/+</td>
<td>±/+</td>
<td>+/++</td>
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<tr>
<td>With halo</td>
<td>±</td>
<td>+/++</td>
<td>-</td>
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<tr>
<td>Granular/clumped</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Cytoplasmic</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>Sites of replication</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tubular/Bowman’s capsule epithelial cells</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>±</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Acute tubular injury</td>
<td>+++</td>
<td>±/+</td>
<td>+/++</td>
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<tr>
<td>Interstitial inflammation</td>
<td>+</td>
<td>+/-</td>
<td>+/++</td>
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<tr>
<td>Focal parenchymal necrosis</td>
<td>±/+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Interstitial hemorrhage</td>
<td>+/+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Granulomatous inflammation</td>
<td>+/+</td>
<td>-/+</td>
<td>+</td>
</tr>
</tbody>
</table>

Modified from Singh and Nickeleit.\(^15\) Pathological features expressed as absent (-), very rare (±), occasional (+), common (++), and frequent (+++).
quent increase in immunosuppression facilitates viral replication and subsequent infection. This presumes viral latency, which is widely appreciated for CMV and polyomavirus, but also occurs with adenovirus. Alternatively, a higher degree of immunosuppression may be used because of an increased immunologic risk as seen in highly sensitized recipients, ABO incompatibility, or a positive crossmatch. Recipients in this situation would be at higher risk for viral reactivation or more aggressive de novo infections, in addition to an increased risk of rejection.

Second is reduced immunosuppression facilitating rejection. The primary treatment for opportunistic viral infections such as adenovirus or polyomavirus is a reduction in immunosuppression, which may precipitate allograft rejection.

**Direct Relationship**

Acute rejection in association with BKV infection is well-recognized, although it remains controversial whether a direct cause and effect relationship exists, that is, whether viral infection precipitates acute rejection. Viral infection of the graft causes both direct (viral cytopathology) and indirect (cell-mediated immunopathology through the host response) tissue injury. This damage and the ensuing immune response may be analogous to the ischemia-reperfusion model of the early posttransplant period. Both activate the innate immune system, through toll-like receptors (TLR2) and other pathways involving cytokines including IL-6, TNF-α, and IFN-γ. Although the subsequent adaptive immune response is largely virus-specific, the component parts of this process (increased antigen presentation, cytokine and chemokine release, and T cell and other cellular proliferation) may amplify graft alloimmunity.

The similar pathologic findings of viral-associated interstitial nephritis and acute rejection complicate the diagnosis and treatment of both processes. The differential diagnosis is particularly problematic in BKV nephropathy in which the nongranulomatous tubulo-interstitial inflammatory response to virus is identical to acute tubulointerstitial rejection. Criteria for diagnosing acute rejection in cases of BKV nephropathy were discussed at the last Banff Conference on Allograft Pathology in 2009. The general consensus was that concomitant rejection can be diagnosed if there are tubulointerstitial rejection changes in a biopsy which are clearly separate from areas of tubular viral infection as demonstrated with SV40 immunostain, if there is an intimal arteritis lesion diagnostic of vascular rejection, or if there is C4d staining in peritubular capillaries indicating a humoral rejection process.

In the case of ADV, the frequently granulomatous character of the inflammatory reaction to virus is more readily distinguished from rejection, as granulomatous inflammation is not a recognized feature of rejection. Nevertheless, acute rejection could still coexist with ADV nephritis. Our case demonstrates findings consistent with ADV infection (palisading tubulo-centric granulomas and tubular viral cytopathic changes) and acute rejection (peritubular capillaritis, tubulitis, and endothelialitis) in the same biopsy. The follow-up biopsy revealed evidence of ongoing rejection, including glomerulitis, but without definite morphologic evidence of a viral infection.

Treatment strategies for ADV allograft infection include reduction of immunosuppression, and specific antiviral therapy. A range of outcomes have been reported, from rapid clearing of the virus and recovery of allograft function to fatal outcomes. The optimal treatment strategy is not clear; our patient received a standard steroid pulse for suspected rejection, followed by a reduction in immunosuppression for confirmed ADV infection, followed by yet another steroid pulse and IVIG for biopsy-proven rejection, and lastly a more sustained reduction in immunosuppression to assist with clearing of the virus. The essentially normal allograft biopsy 6 weeks after the first biopsy demonstrates a remarkable recovery from both ADV and/or rejection-related inflammatory injury, and highlights the potential for good long term prognosis of ADV nephritis after viral clearance compared with the often protracted infection of polyomavirus nephropathy with associated chronic graft injury.

**DISCLOSURES**

None.

**REFERENCES**


