

# Cytomegalovirus-Induced Necrotizing and Crescentic Glomerulonephritis in a Renal Transplant Patient

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● A 35-year-old black man with end-stage renal disease from biopsy-proven focal segmental glomerulosclerosis developed worsening function of his renal allograft 160 days after living related donor renal transplantation. Renal biopsy showed necrotizing and crescentic glomerulonephritis (NCGN) and presence of intraglomerular viral inclusions confirmed by immunocytochemical stain and in situ hybridization techniques to be cytomegaloviral in origin. Electron microscopy showed no immune complexes, and workup for other causes of NCGN was negative. The patient was treated with ganciclovir without other changes in his immunosuppressive regimen. After 8 weeks of ganciclovir therapy, a second renal transplant biopsy showed resolution of the glomerular process and disappearance of the cytomegalovirus (CMV) inclusions. The resolution of the glomerular process with treatment for CMV infection, and without other change in therapy, strongly supports a causative link between CMV and NCGN in this patient. This case represents the first report of CMV-associated NCGN in a renal transplant patient.

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**INDEX WORDS:** Kidney transplantation; cytomegalovirus infections; glomerulonephritis.

**C**YTOMEGALOVIRUS (CMV) infection in renal transplant patients is associated with a variety of well-defined clinical consequences. However, documentation of an association between CMV infection and glomerular disease of the transplanted kidney has been difficult to confirm, as addressed in a recent review.<sup>1</sup> We report the case of a renal transplant patient who developed necrotizing and crescentic glomerulonephritis (NCGN) with accompanying intraglomerular cytomegalic inclusion bodies in the transplant kidney. Similar renal pathology has been reported in infants with congenital cytomegalic inclusion disease<sup>2-4</sup>; however, no prior case of CMV-induced NCGN has been reported in a renal transplant patient.

## CASE REPORT

A 35-year-old black man with end-stage renal disease secondary to biopsy-proven focal segmental glomerulosclerosis underwent living related donor renal transplantation 9 months after initiation of dialysis. The donor kidney was from his six-antigen-mismatched, CMV-positive sister. Pretransplantation assessment of the patient included demonstra-

tion of negative serologies for CMV, hepatitis B, hepatitis C, toxoplasma, and human immunodeficiency virus (HIV). Epstein-Barr virus (EBV) antibody panel showed evidence of remote EBV infection. Medical history was otherwise remarkable only for reactive airway disease.

Posttransplantation, the patient received OKT3 induction therapy 5 mg daily for 7 days. Intravenous ganciclovir was administered daily during the first 7 days. He was discharged on posttransplantation day 7 on prednisone 0.5 mg/kg/d, azathioprine 1.5 mg/kg/d, cyclosporine 8 mg/kg/d, and acyclovir 800 mg three times daily. Serum creatinine (Cr) on the day of discharge was 1.9 mg/dL. The Cr stabilized in the 2.0 to 2.5 mg/dL range with absence of proteinuria on urinalysis.

Forty-five days posttransplantation the serum Cr rose to 2.9 mg/dL in the absence of signs or symptoms of illness. Cyclosporine levels were therapeutic, and urinalysis showed 2+ protein. Renal ultrasound showed no evidence of obstruction, and resistive indices were within normal limits. Percutaneous renal biopsy could not be performed because of bowel overlying the upper pole of the kidney and close proximity of the iliac vessels posterior to the lower pole. After three doses in 3 days of 500 mg intravenous solumedrol, serum creatinine was 3.4 mg/dL. The patient was then treated with OKT3 and simultaneous ganciclovir therapy. On completion of a 7-day course of OKT3, serum Cr was 2.0 mg/dL. The patient was discharged on mycophenolate mofetil 1.0 g twice daily, cyclosporine, prednisone, and acyclovir 800 mg three times daily. Because of his high risk of CMV infection, especially after treatment with OKT3 for presumed rejection, CMV immune globulin was initiated on day 50 at a dosage of 150 mg/kg and was given again 2, 4, 6, 10, and 14 weeks from initiation.

The patient did well until 138 days posttransplantation, when he developed cough, rhinorrhea, diarrhea, and postural hypotension. He was afebrile, and transaminases, bilirubin, and white blood cells (WBC) remained within normal limits. Chest x-ray was unremarkable, and serum Cr was 2.2 mg/dL. Urinalysis now showed 3+ protein. His symptoms gradually resolved over a 10-day period with no specific therapy. The patient remained on acyclovir and CMV immune globulin and was doing well until day 160 posttransplantation, when the serum Cr rose to 3.0 mg/dL with

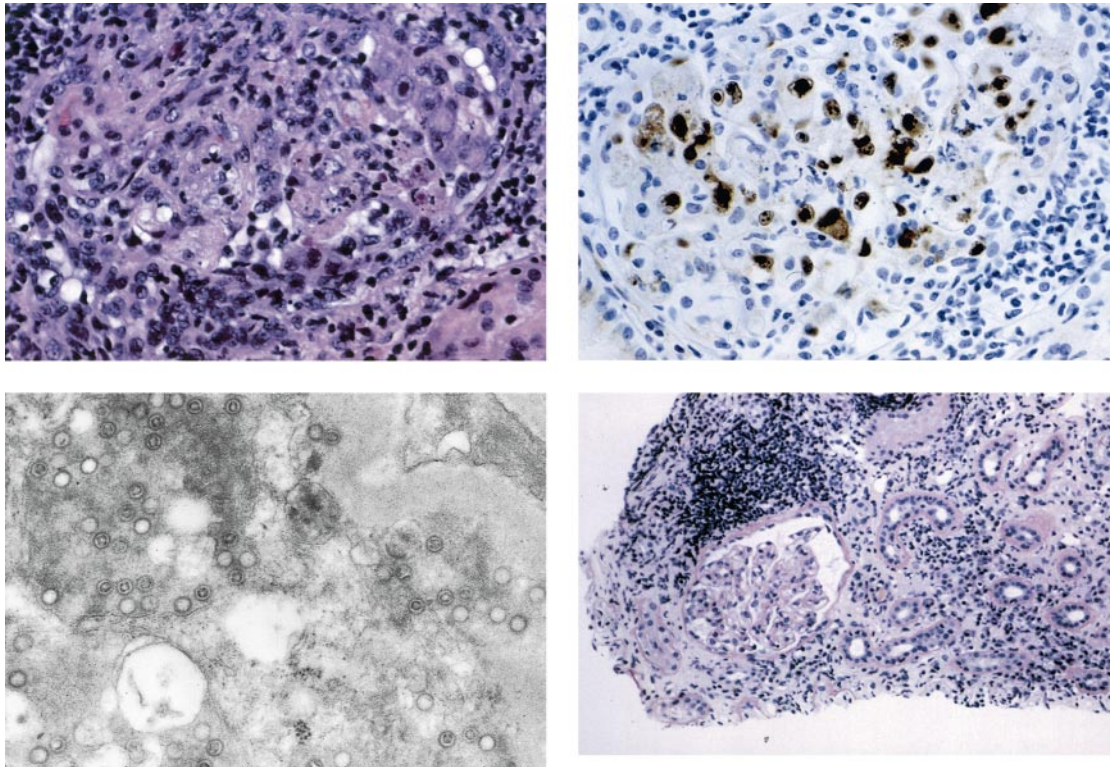
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**Fig 1.** (Top left) High-power view of a representative glomerulus with cellular proliferation, karyorrhexis, and cellular crescent formation. A prominent intranuclear inclusion morphologically consistent with CMV is present in the 2 o'clock position (Hematoxylin-eosin stain; original magnification  $\times 400$ ).

**Fig 2.** (Top right) Glomerulus stained with immunostain specific for CMV showing positive staining of numerous intranuclear and intracytoplasmic CMV viral inclusions (CMV immunostain; original magnification  $\times 400$ ).

**Fig 3.** (Bottom left) Ultrastructural examination of a glomerulus showing endothelial cytoplasmic viral inclusions measuring approximately 80 to 100 nm, and some with a central electron-dense round core and binding envelope (Lead citrate and uranyl acetate stain; original magnification  $\times 82,240$ ).

**Fig 4.** (Bottom right) Medium-power view of renal parenchyma with patchy mononuclear inflammation and associated focal tubulitis. The glomerulus showed no evidence of hypercellularity, necrosis, karyorrhexis, or crescent formation (Hematoxylin-eosin stain; original magnification  $\times 200$ ).

urinary protein still 3+ by dipstick analysis. The patient was without symptoms and afebrile, and the liver function tests remained normal. WBC had dropped to 3,800 cells/ $\mu$ L. Percutaneous renal biopsy was again technically impossible, and an open surgical biopsy was performed.

Light microscopic examination showed approximately 100 glomeruli with diffuse endocapillary hypercellularity, which varied in extent both focally and segmentally. Associated focal segmental necrosis and cellular crescent formation were noted in approximately 30% of the glomeruli, including several with circumferential cellular crescent formation and disruptions in Bowman's capsule (Fig 1). The most striking glomerular feature was the presence of numerous viral inclusions morphologically consistent with cytomegalovirus, seen both on the hematoxylin-eosin-stained sections and by a special immunocytochemical stain for cytomegalovirus (Fig 2). The inclusions involved both endothelial cells and rare interstitial cells. There was interstitial infiltration by mononuclear leukocytes composed predominantly of variably transformed lymphocytes involving at

least 50% of the renal parenchyma. There was an associated focal tubulitis with four to six lymphocytes/tubular cross section. No significant sclerotic, necrotizing, or inflammatory changes were noted in arterioles or small arteries. Direct immunofluorescent microscopic examination showed 30 glomeruli with low-intensity (1 to 2+) irregular staining with antisera specific for immunoglobulin (Ig) G, IgM, IgA, C3, C1q, and kappa and lambda light chains. More intense focal irregular staining was seen for antiserum specific for fibrin. Ultrastructural examination showed extensive mesangial matrix expansion and glomerular basement membrane collapse. Fibrin tactoids were noted in some capillary lumens. No well-defined immune complex-type electron-dense deposits were identified. Endothelial cytoplasmic viral inclusions measuring approximately 80 to 100 nm were noted, some with a central electron-dense round core and a binding envelope (Fig 3). Renal biopsy tissue from the paraffin block was used for in situ hybridization studies and was strongly positive for CMV. In situ hybridization studies for EBV EBER 1, herpes simplex viruses I and II, and adenovirus

were negative. Immunocytochemistry for polyoma virus protein was negative. All studies had adequate positive and negative controls.

Workup after the biopsy results included demonstration of positive Shell vial and routine viral blood cultures for CMV and presence of CMV pp65 antigen in white blood cells from peripheral blood using a fluorescent monoclonal antibody technique. Anti-neutrophil cytoplasmic autoantibodies (cANCA and pANCA) were negative by immunofluorescence and enzyme-linked immunosorbent assay (ELISA) methods. An HIV test was repeated and confirmed to be negative. EBV serologies were essentially unchanged.

The patient was initiated on intravenous (IV) ganciclovir followed by a 2-month period of oral ganciclovir. He remained on the same doses of mycophenolate mofetil, cyclosporine, and prednisone. The patient noted an overall improvement in his energy level but was otherwise symptomatically unchanged. Serum creatinine stabilized in the 2.3 to 2.7 mg/dL range.

Eight weeks after initiation of ganciclovir treatment, a repeat renal biopsy was performed. Light microscopic examination of the second biopsy showed 14 glomeruli for evaluation that had no significant abnormalities, including no hypercellularity, necrosis, karyorrhexis, crescent formation, sclerosis, or adhesions to Bowman's capsule (Fig 4). No viral cytopathic inclusions compatible with CMV were identified. Rare foci of tubulitis with fewer than four cells/tubular cross section associated with sparse patchy mononuclear inflammatory cell infiltrates were seen. Blood vessels showed no evidence of sclerotic or inflammatory changes. Direct immunofluorescent microscopic examination showed three glomeruli with low-intensity (1+) focal mesangial staining for antisera specific for IgM and C3 and no glomerular or extraglomerular staining with antisera specific for IgG, IgA, C1q, fibrin, or kappa or lambda light chains. No glomeruli were available in the tissue submitted for ultrastructural examination.

## DISCUSSION

The reported case is characterized by the unique combination of NCGN and intraglomerular cytomegalic inclusions in a renal allograft. A causative link between CMV infection and the glomerular process was suspected based on the pathology findings and the knowledge that similar glomerular findings have been observed in infants with cytomegalic inclusion disease.<sup>2-4</sup>

Before accepting this association, we investigated other potential causes of NCGN. The patient's primary renal disease was biopsy-proven focal and segmental glomerulosclerosis, a lesion not known to recur as, or evolve to, NCGN. There was no evidence of a systemic vasculitis, systemic lupus erythematosus, pulmonary renal syndrome, or active infection other than CMV. This included laboratory demonstration of absence of, or negative tests for, pANCA, cANCA,

anti-nuclear antibody (ANA), anti-glomerular basement membrane antibodies, HIV, hepatitis B, hepatitis C, and rapid plasma reagin (RPR). An underlying immune complex-mediated renal disease is unlikely in view of the absence of immune deposits on electron microscopy.

The pathological finding of extensive tubulointerstitial inflammation surrounding the affected glomeruli in a nodular pattern raised the possibility of an underlying posttransplantation lymphoproliferative disorder (PTLD). Although biopsy material showed no immunoblastic nuclear atypia or areas of serpiginous necrosis, polymorphic PTLD lesions, particularly those in a resolving phase, may not show these characteristic features.<sup>5</sup> We therefore pursued *in situ* hybridization techniques looking for evidence of EBV genomes. *In situ* hybridization in this case was negative for EBV EBER 1, adenovirus, and herpes simplex I and II viruses, but was strongly positive for CMV in glomerular and interstitial cells. EBV serology were essentially unchanged from pretransplantation levels, and there was no examination or radiographic evidence of lymphadenopathy. In view of this combination of findings, it was concluded that the patient's illness was not consistent with PTLD.

In the absence of an alternative explanation for this patient's glomerulonephritis, ganciclovir treatment was initiated on the assumption that the process was CMV related. No change in immunosuppressive medications or other changes in therapy were made. The clinical and pathological resolution of glomerulonephritis and tubulointerstitial inflammation after initiation of ganciclovir further supports the contention that this patient's renal disease was CMV mediated.

There is controversy in the literature as to whether CMV infection can cause glomerular disease in renal allografts. In 1981, Richardson et al<sup>6</sup> reported a glomerular lesion in four renal transplant patients characterized by distended, occluded capillary loops with endothelial cell swelling or necrosis and accumulation of mononuclear cells and fibrillary material in glomerular capillaries. There was very little or no increase in mesangial cellularity, and no cellular crescents were observed. The observation that this lesion was present only in patients with CMV viremia, that one patient had positive immunofluorescence staining for CMV antigens in glomerular

capillary walls, and that two allografts recovered renal function after reduction in immunosuppression suggested to the authors that this process was a CMV-associated glomerulopathy. Further cases of this proposed transplant CMV-associated glomerulopathy were reported.<sup>7,8</sup> Tuazon et al<sup>8</sup> suggested that the association with CMV infection may be indirect, immune mediated, and that CMV infection may be neither necessary nor sufficient for its development. Subsequently, others have questioned the validity of any association between CMV infection and this lesion based on the observation that similar glomerular findings occur in the absence of evidence of CMV infection.<sup>9-11</sup> In some of these studies, the term *transplant glomerulopathy* has been preferred over CMV glomerulopathy, emphasizing the lack of a clear causative relationship with CMV.<sup>9,10</sup>

The current case is distinct from transplant glomerulopathy based on several observations, including (1) the pathology in this case is that of crescentic and necrotizing glomerulonephritis, a lesion clearly pathologically different from transplant glomerulopathy, (2) intraglomerular cytomegalic inclusion bodies were present and abundant in this case and were not reported in transplant glomerulopathy, and (3) this case showed a clear clinical and pathologic response to therapy directed specifically toward cytomegalovirus infection in the absence of confounding variables and changing immunosuppressive dosing.

CMV-associated glomerulopathy is not the only glomerular lesion described in CMV infected renal transplants. Rao et al<sup>12</sup> reported a case in which treatment of CMV infection was associated with resolution of immunotactoid glomerulopathy in a renal allograft. Our patient had no immunotactoid fibrils or pathological findings suggestive of immunotactoid glomerulopathy.

Though it has previously been difficult to prove an association between CMV infection and glomerular disease in renal transplant patients, CMV infection has been clearly linked to glomerular disease in nontransplant patients. Children with congenital cytomegalic inclusion disease have been reported to develop proliferative glomerulonephritis.<sup>2-4</sup> In some of these infants, pathology findings of NCGN with intraglomerular cytomegalic inclusions in the glomeruli were

noted, a pathologic pattern very similar to the current case.<sup>3,4</sup> In an adult patient with disseminated CMV infection and focal proliferative glomerulonephritis, Ozawa and Stewart<sup>13</sup> reported the presence of glomerulus-bound antibodies directed to CMV antigen. In a murine model, animals treated with antilymphocyte globulin (ALG) and subsequently infected with CMV developed a proliferative glomerulonephritis with crescent formation.<sup>14</sup> Thus, the evidence in the nontransplant setting in humans and animals suggests that NCGN due to CMV infection would be the expected glomerular lesion observed in transplants.

The mechanism by which CMV infection might induce a NCGN in this patient is speculative. Absence of intraglomerular immune complexes, antiglomerular basement membrane antibodies, or ANCA suggests direct injury to glomerular cells as the most likely pathophysiologic mechanism of injury. In vitro study of human kidney cell lines has confirmed that all cells within the kidney, including glomerular endothelial, epithelial, and mesangial cells, can be infected by CMV.<sup>15</sup> Grefte et al<sup>16</sup> have shown the existence of circulating CMV-infected endothelial cells and have suggested the possibility that widespread vascular injury may be induced by CMV infection. Beneck et al<sup>4</sup> confirmed the presence of viral particles in the nucleus and cytoplasm of a patient with proliferative glomerulonephritis. Intraglomerular cytomegalic inclusion bodies without glomerulonephritis were observed in a patient with CMV-associated tubulointerstitial nephritis.<sup>17</sup> In transplant biopsy specimens, in situ hybridization techniques have previously shown that interstitial cells are the primary target for CMV in the transplant kidney; however, some glomerular cells also may be infected.<sup>18</sup> A transplant patient with recurrent membranoproliferative glomerulonephritis and active CMV infection was noted to have intraglomerular endothelial cytomegalic inclusions, which resolved with ganciclovir therapy.<sup>1</sup>

Thus, the evidence from this case and from the literature suggests that glomerular endothelial cells can, and sometimes do, become infected with CMV. We would speculate that, in this patient, the glomerular endothelial cell infection led to cellular injury and ultimately to NCGN. Why this patient was unique to the transplant

experience and developed what appears to be the first reported case of CMV-induced NCGN in a transplanted kidney is unclear. True confirmation of a causative association between CMV infection and NCGN in transplant patients will require observation of similar occurrences in other patients.

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