Kidney Medicine

Monoclonal IgG4/2κ Deposition Following Eculizumab Therapy for Recurrent Atypical Hemolytic Uremic Syndrome in Kidney Transplantation

Priyamvada Singh, Hui Chen, Craig E. Gordon, Sandeep Ghai, J. Mark Sloan, Karen Quillen, Sara Moradi, Vipul Chitalia, Amitabh Gautam, Joel Henderson, and Jean M. Francis

Eculizumab is an emerging therapy for atypical hemolytic uremic syndrome (aHUS). Early identification and treatment of recurrent aHUS after kidney transplantation requires a high clinical suspicion but results in improved graft function and patient outcome. We present a patient who developed recurrent aHUS after kidney transplantation that responded to eculizumab therapy. A kidney biopsy was performed to confirm resolution of thrombotic microangiopathy 8 weeks after eculizumab treatment initiation and revealed no features of thrombotic microangiopathy. Instead, the biopsy revealed monoclonal immunoglobulin G (IgG)4/2κ deposition in the glomerular tufts, vasculature, and atrophic tubular basement membranes. IgG4/2κ deposits are a rare pathologic finding following eculizumab therapy, and the long-term effect of these deposits on kidney function remains unknown.

Complete author and article information provided before references.

Correspondence to J. Henderson (jmhender@bu.edu) or J.M. Francis (jean.francis@bmc.org)

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INTRODUCTION

Thrombotic microangiopathy (TMA) is a clinical syndrome that manifests with microangiopathic hemolytic anemia, thrombocytopenia, and end-organ damage secondary to arteriolar and capillary microthrombi and endothelial injury.[1] Atypical hemolytic uremic syndrome (aHUS) is a rare form of TMA caused by overactivity of the alternative pathway of complement, with high rates of morbidity and mortality.[1] Genetic anomalies in the alternative pathway of complement are seen in 60% of cases of aHUS and are responsible for excessive complement activation.[2]

The risk for aHUS recurrence after kidney transplantation varies by complement mutation. Mutations affecting different complement system proteins (C3 and complement factor B [CFB]) or complement regulatory proteins have been identified to be associated with aHUS.[2,3] Mutations affecting complement factor H (CFH) are the most common and are noted in 30% of the cases, whereas mutations in the gene encoding thrombomodulin (THBD) are the least common and are seen in only 3% to 5% of individuals with aHUS.[2,3] Other mutations have been described to affect membrane cofactor protein, factor I, CFB, and C3.[2,3] A genotype-phenotype correlation is seen, and a specific genetic mutation is associated with the severity of the disorder and the likelihood of response to treatment. For example, membrane cofactor protein mutations have lower risk for permanent kidney damage and recurrence following kidney transplantation.[2,3]

Eculizumab is approved by the US Food and Drug Administration for use in aHUS. It is a humanized mouse monoclonal antibody formed by replacement of the heavy chain constant region of the parent antibody by components of both human immunoglobulin G2 (IgG2) and IgG4 and thus lacks the ability to activate complement.[1,4,5] It recognizes the human complement protein C5 and blocks the cleavage of C5 to C5a and C5b, thus preventing induction of the terminal complement cascade, including the formation of the membrane attack complex (C5b-9).[4-6]

We present a case of de novo monoclonal antibody deposition in the kidney, restricted to the IgG2 and IgG4 subclasses following eculizumab therapy, in association with de novo positive monoclonal antibody on serum immunofixation.

CASE REPORT

A 22-year-old man with chronic kidney disease (CKD) stage 5 of unknown cause underwent preemptive living related kidney transplantation with a kidney from his 26-year-old sister. His CKD was first noted at age 12 years, but no specific diagnosis was made. We did not perform kidney biopsy due to his advanced CKD. Family history was significant for a few renal cysts in his father with no hypertension or decreased kidney function, nephrectomy for an unknown reason in his uncle, and CKD in his brother. He has 6 brothers and 5 sisters, and other than the brother with CKD, all were presumably healthy. Because the cause of his kidney disease was unknown, we tested for possible aHUS before his transplantation; lactate dehydrogenase level was 229 (reference range, 171-308) U/L, platelet count was 219 (reference range, 150-400) K/μL), and peripheral smear and haptoglobin were normal. Genetic testing for complement cascade showed the patient to be heterozygous for the CFH C>T promoter polymorphism. This polymorphism is common in the healthy population (∼28%) but statistically enriched in the aHUS population. His sister had normal donor workup results per our center's protocol.

The donor nephrectomy surgery on the patient's sister was uneventful. The surgical course for the kidney

implantation was routine and uneventful. He was started on antithymocyte globulin (weight-based protocol, 1.5 mg/kg) and steroid taper for induction. The recipient's postoperative course was complicated by thrombocytopenia on day 3 after transplantation (nadir platelet count, 36 K/μL) and delayed graft function (creatinine levels days 0, 1, 2, and 3 were 5.8, 5.9, 6, and 6.2 mg/dL, respectively). We did not initiate treatment with tacrolimus or other calcineurin inhibitors due to concerns of delayed graft function. Testing revealed an elevated lactate dehydrogenase level (673 [reference, 171-308] U/L), low haptoglobin level (<7 [reference, 44-184] mg/dL), and schistocytes on a peripheral-blood smear. Donor-specific antibody was negative. Our medical center's multidisciplinary TMA consult team[7] directed the care and rapidly arrived at a diagnosis. Eculizumab treatment was initiated for presumed recurrent aHUS on day 3 posttransplantation. A kidney biopsy performed on day 5 posttransplantation following the recovery of thrombocytopenia confirmed the diagnosis of acute TMA without evidence of T-cell or antibody-mediated rejection (Fig 1).

All hematologic parameters improved rapidly following eculizumab treatment and the patient's kidney function slowly improved: serum creatinine level decreased from 4.5 to 2.8 mg/dL, with no proteinuria over a period of 8 weeks. After treatment with eculizumab was begun, evidence of effective and sustained terminal complement inhibition was observed, with 50% hemolytic complement (CH50) levels consistently <10 complement activity enzyme (CAE) units, suggestive of a complete blockade of the terminal complement pathway.

A repeat kidney biopsy was performed because serum creatinine level remained at ~2.8 mg/dL and did not decline further, as expected. The biopsy was performed 7 weeks after transplantation and after 5 doses of eculizumab were administered. The biopsy demonstrated complete resolution of TMA features on a background of essentially normal renal parenchyma at the light microscopic level (Fig 2). The degree of glomerulosclerosis and tubulointerstitial scarring was the same, with ~3% global glomerulosclerosis and <10% tubulointerstitial scarring. Overall, immunofluorescence staining patterns and intensity for C3 in glomeruli were similar in the pre- and posttreatment biopsies (2 to $3^+/4^+$). However, immunofluorescence microscopy revealed the unexpected finding of weak to moderate monoclonal reactivity for IgGκ segmentally in glomeruli and focally in vasculature and basement membranes of atrophic tubules, with no corresponding staining for λ light chain (Fig 2).

IgG subtype staining revealed the IgG deposits to exhibit $2^+/4^+$ reactivity for IgG4, $1^+/4^+$ reactivity for IgG2, and no reactivity for IgG1 or IgG3 (Fig 3). Electron microscopy did not reveal electron-dense deposits corresponding to the pattern of distribution on immunofluorescence. Given the deposition and staining patterns of the deposits, it is most likely that these deposits reflect the binding of eculizumab to complement proteins in the kidney.

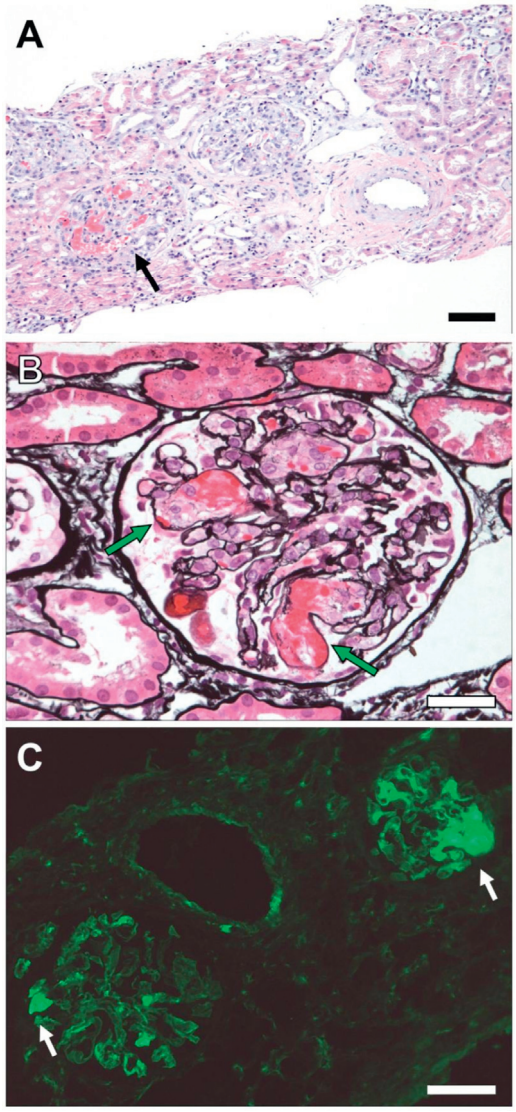

Figure 1. Morphologic findings in the allograft biopsy at 5 days posttransplantation. (A) Brightfield microscopy reveals the focal acute tubular injury, evidenced by focal tubular luminal distension, and tubular epithelial flattening and vacuolization, minimal endocapillary inflammation in glomeruli (Banff score g0-1), and focal glomeruli exhibiting red blood cell stasis (arrow). The biopsy otherwise shows well-preserved parenchyma and no evidence of rejection. Small arteries are unremarkable and show no evidence of endothelialitis, vasculitis (Banff score v0), or thrombosis (hematoxylin and eosin; bar = 100 μm). (B) Higher magnification brightfield microscopy of Jones' methenamine silver–stained section reveals focal segmental glomerular capillary occlusion by fibrinoid material, red blood cells and red blood cell fragments, and karyorrhectic debris, indicative of microthrombus formation (green arrows) (bar = 50 μm). (C) Immunofluorescence microscopy for fibrinogen reveals segmental immunoreactivity in glomeruli (arrows), indicative of the presence of fibrin microthrombi (bar = 100 μm).

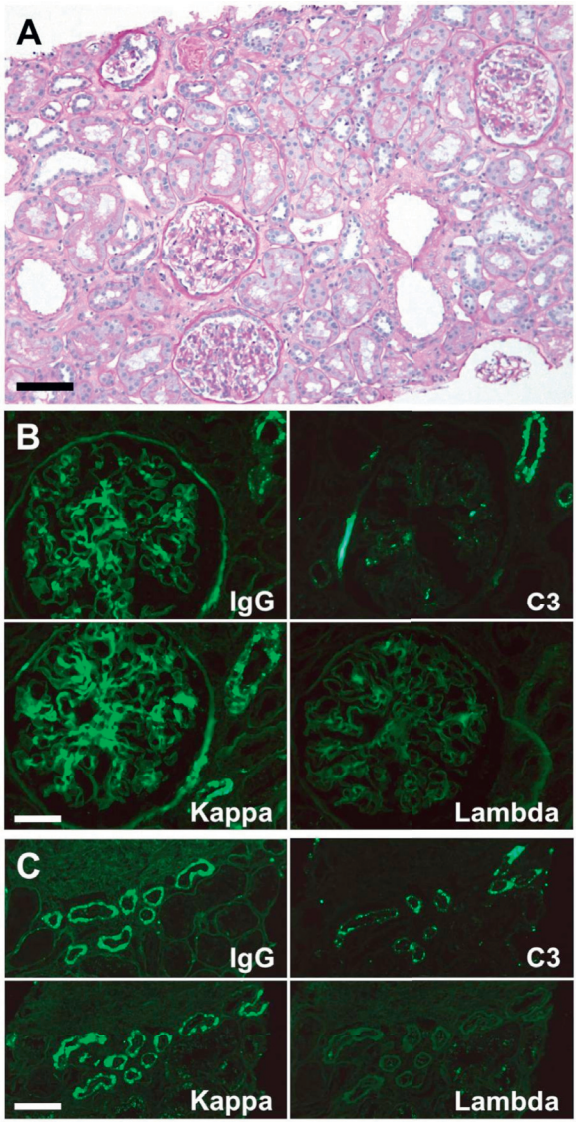

Figure 2. Morphologic findings in the allograft biopsy at 7 weeks posttransplantation. (A) By brightfield microscopy, the parenchyma is generally well preserved and there is no evidence of rejection. Small arteries are unremarkable and show no evidence of endothelialitis, vasculitis, thrombosis, or fibrosis. There are focal tubular atrophy and interstitial fibrosis (top left of image), and rare tubules contain granular debris. All glomeruli shown are preserved but show mild mesangial expansion; 1 sclerosed glomerulus was evident in the sample processed for immunofluorescence (not shown) (periodic acid–Schiff stain; bar = 100 μm). (B) Immunofluorescence microscopy reveals segmental immunoreactivity for immunoglobulin G (IgG; $2^+/4^+$), C3 (trace to $2^+/4^+$), and κ light chain ($2^+/4^+$ to $3^+/4^+$) in the mesangium of viable glomeruli and arterioles. A sclerosed glomerulus showed similar but stronger immunoreactivity for IgG and κ light chain (not shown). IgA, IgM, λ light chain, and C1q were essentially negative throughout the biopsy except for irregular nonspecific staining for IgM (2^+), λ (trace), and C1q (trace, dull) in the sclerosed glomerulus (not shown) (bar = 50 μm). (C) By immunofluorescence microscopy, a focus of tubulointerstitial chronic damage reveals mild to moderate immunoreactivity for IgG, C3, and κ light chain in basement membranes of atrophic tubules. Adjacent viable tubules are negative (bar = 100 μm).

The patient's creatinine level now has stabilized between 1.5 and 1.7 mg/dL. The future plan is to stop tacrolimus treatment gradually after starting belatacept therapy and take him off the eculizumab treatment. At this time, multiple attempts have been made to space the eculizumab dosing, but he always exhibits signs of recurrent TMA with a decrease in platelet count, elevated lactate dehydrogenase level, and a significant decrease in haptoglobin level.

DISCUSSION

aHUS is a rare disorder (2 per million incidences in the United States) that frequently recurs after kidney transplantation.[1,2] Monoclonal murine antibodies directed at human C5 effectively prevent the generation of chemotactic C5a and the formation of the membrane attack complex (C5b-9).[5] Eculizumab is the humanized form of these monoclonal antibodies and is formed by the fusion of C5-specific variable κ light chain region to a hybrid heavy chain created from portions of IgG2 and IgG4. The IgG2 constant region 1 and IgG4 constant regions 2 and 3 were selected for their inability to activate complement.[5] The presence of immunostaining exclusively for IgG2, IgG4, and κ light chain would thus be expected if eculizumab were being deposited in tissues.

A unique finding in our case was the presence of unexpected de novo monoclonal immune-protein deposits in the kidney. The deposits exhibited immunoreactivity for IgGκ with restriction to the IgG2 and IgG4 subclasses of the γ heavy chain, and an absence of immunoreactivity for all other heavy chain classes and λ light chain, suggesting the binding of monoclonal eculizumab to complement in renal tissues. A similar IgGκ with restricted IgG subtype deposits was reported in post–eculizumab treatment biopsies in 3 patients with dense deposit disease and 2 with C3 glomerulonephritis[8-10] and 3 patients with high donor-specific antibody levels.[11] To our knowledge, this is the first case showing deposition of the de novo monoclonal staining for IgGκ in aHUS.

The distribution and characteristics of the IgGκ binding are similar to immune complex–mediated glomerulonephritis or monoclonal immunoglobulin deposition disease but should not be misinterpreted as a transformation to either.[8,9] Studies have examined the long-term use of eculizumab in patients with paroxysmal nocturnal hemoglobinuria and found no evidence of the development of proteinuria and decreased kidney function that is typical of monoclonal immunoglobulin deposition disease.[12,13] Mild chronic damage was noted in the follow-up biopsy, and relatively prominent IgG-K deposits were observed in atrophic tubules and sclerosed glomeruli (Figs 2 and 3).

The cause of the C3 deposits seen on biopsy is uncertain. It is common, particularly in the transplantation setting in which glomerular hyperfiltration is underway, for nonspecific glomerular C3 deposition to be observed. In this case, similar glomerular C3 intensity was seen in

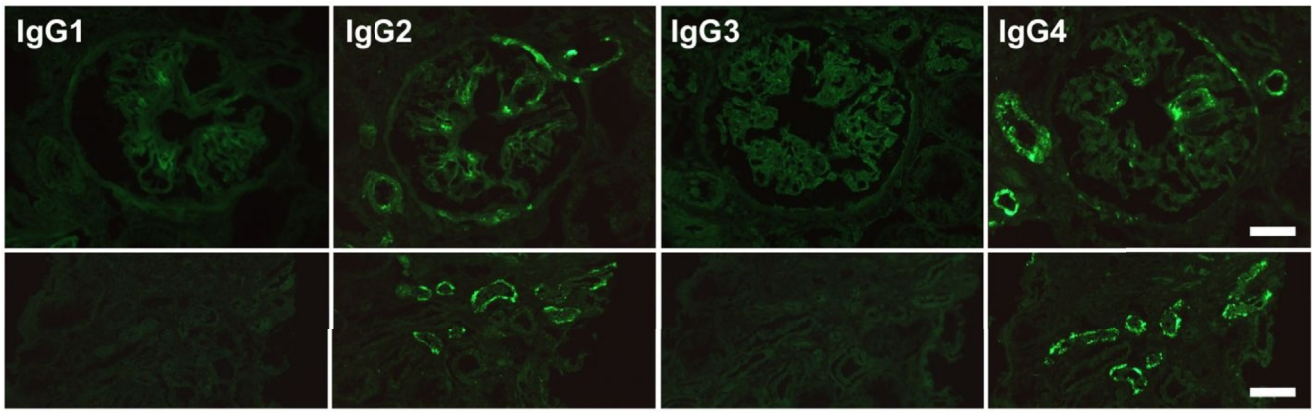

Figure 3. Immunoglobulin G (IgG) subtype staining of allograft biopsy at 7 weeks posttransplantation. (Top row) Segmental immunoreactivity for IgG2 ($1^+/4^+$) and IgG4 ($2^+/4^+$) in a viable glomerulus and adjacent arterioles (bar = 50 μm). (Bottom row) Immunoreactivity for IgG2 and IgG4 in basement membranes of atrophic tubules (same focus as in Fig 2). IgG1 and IgG3 are negative (bar = 100 μm).

both biopsy specimens (before and after eculizumab treatment), suggesting that the C3 deposition is unrelated to eculizumab treatment. Nevertheless, other mechanisms of C3 deposition also warrant consideration. We did not identify any known complement mutations in our patient as a cause of the aHUS. It is possible that our patient has a currently unknown mutation leading to proximal complement activation of the alternative pathway. Eculizumab would only be expected to block terminal complement and therefore it is possible that this could explain the C3 deposits.

Another possibility is that the allograft was recovering from acute kidney injury and diffuse endothelial damage, likely leading to ongoing complement activation and C3 deposition. Another possibility is that eculizumab activates the proximal lectin complement pathway. It remains to be seen whether the presence of such deposits could play a role in the development of chronic kidney damage. The clinical significance of eculizumab binding to kidney tissue is unclear and mandates closer examination.

ARTICLE INFORMATION

Authors' Full Names and Academic Degrees: Priyamvada Singh, MD, Hui Chen, MD, Craig E. Gordon, MD, Sandeep Ghai, MD, J. Mark Sloan, MD, Karen Quillen, MD, Sara Moradi, MD, Vipul Chitalia, MD, Amitabh Gautam, MD, Joel Henderson, MD, and Jean M. Francis, MD.

Authors' Affiliations: Renal Section, Department of Medicine, Boston University School of Medicine, Boston, MA (PS, CEG, SG, VC); Transplant Nephrology Section, Department of Medicine, Ohio State University, Columbus, OH (PS); Department of Pathology & Laboratory Medicine (HC, SM, JH, JMF), Hematology, Oncology Section, Department of Medicine (JMS, KQ), Whitaker Institute (VC), and Department of Surgery (AG), Boston University School of Medicine, Boston, MA.

Address for Correspondence: Jean M. Francis, MD, Boston University School of Medicine, 650 Albany St, EBRC Bldg, 5th Fl, Boston, MA 02118. (e-mail: jean.francis@bmc.org) or Joel Henderson, MD, Department of Pathology & Laboratory Medicine, Boston University School of Medicine, 670 Albany St, Rm 347, Boston, MA 02118. (e-mail: jmhender@bu.edu)

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