Eculizumab and Complement Activation in Anti—glomerular Basement Membrane Disease

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INTRODUCTION

Anti—glomerular basement membrane (anti-GBM) disease is characterized by pathogenic autoantibodies targeting the α-3 chain of type IV collagen located in the glomerular and alveolar basement membranes. Clinically, it causes rapidly progressive glomerulonephritis and pulmonary hemorrhage. Observational data and mechanistic rationale have largely informed current treatment strategies, including plasmapheresis, glucocorticoids, cyclophosphamide, and rituximab. However, overall and renal prognosis remain guarded, and advancements in therapy are needed.

Emerging data have implicated the complement system in the pathogenesis of anti-GBM disease.[1–5] Eculizumab is an anti-C5 monoclonal antibody that blocks the cleavage of C5, which prevents the formation of C5a, the potent leukocyte chemoattractant, and C5b, the initial reagent in the formation of the membrane attack complex (MAC; also known as C5b-9). This provides an immediate inhibition of the downstream proinflammatory and cytotoxic sequelae of the complement system.

Here, we report the use of eculizumab as rescue therapy in 2 patients with progressive anti-GBM disease on standard therapies, which, to our knowledge, has not been reported to date. We also present kidney biopsy results with extensive complement staining that add to a limited but growing body of literature on the possible role of complement in the renal damage from anti-GBM disease.

CASE PRESENTATION

Case 1

A 52-year-old man presented with 2 months of night sweats, fevers, unintentional weight loss, arthralgias, myalgias, and nonbloody cough. His serum creatinine (SCr) was 1.2 mg/dl. Urinalysis was significant for blood (3+) and protein (2+), with numerous red blood cell casts on sediment analysis. Two weeks later, his SCr was 2.1 mg/dl. Test results for anti-GBM were positive at 133 RU/ml (negative <20), and test results for myeloperoxidase–antineutrophil cytoplasmic antibody (MPO-ANCA) were positive at 109 units (negative <2.8). Given the patient's clinical findings and positive serologic testing, a kidney biopsy was deferred. A diagnosis of dual anti-GBM disease and ANCA-associated vasculitis was made. The patient was hospitalized and received daily plasmapheresis, methylprednisolone 1000 mg i.v. daily for 3 days followed by a prednisone taper, oral cyclophosphamide initially dosed at 2.5 mg/kg per day with adjustments made for renal function, and rituximab (Figure 1a). His SCr continued rising to a zenith 3.9 mg/dl, and his urine output fell from 2000 ml per day to 800 ml per day. The patient received eculizumab 300 mg i.v. after his fifth plasmapheresis session, 600 mg i.v. after his sixth session, and 900 mg i.v. after his tenth plasmapheresis session. His SCr subsequently began to improve. At last follow-up 3 years after initial presentation, his SCr was at his new baseline of 1.9 mg/dl with absent hematuria, and his anti-GBM and ANCA titers remained undetectable. The circulating levels of anti-GBM antibody are depicted in Supplementary Figure S1. The patient continues to receive maintenance of remission therapy with rituximab. His course was complicated by asymptomatic late-onset neutropenia from rituximab that resolved with filgrastim, and dermatomal shingles that resolved with valacyclovir.
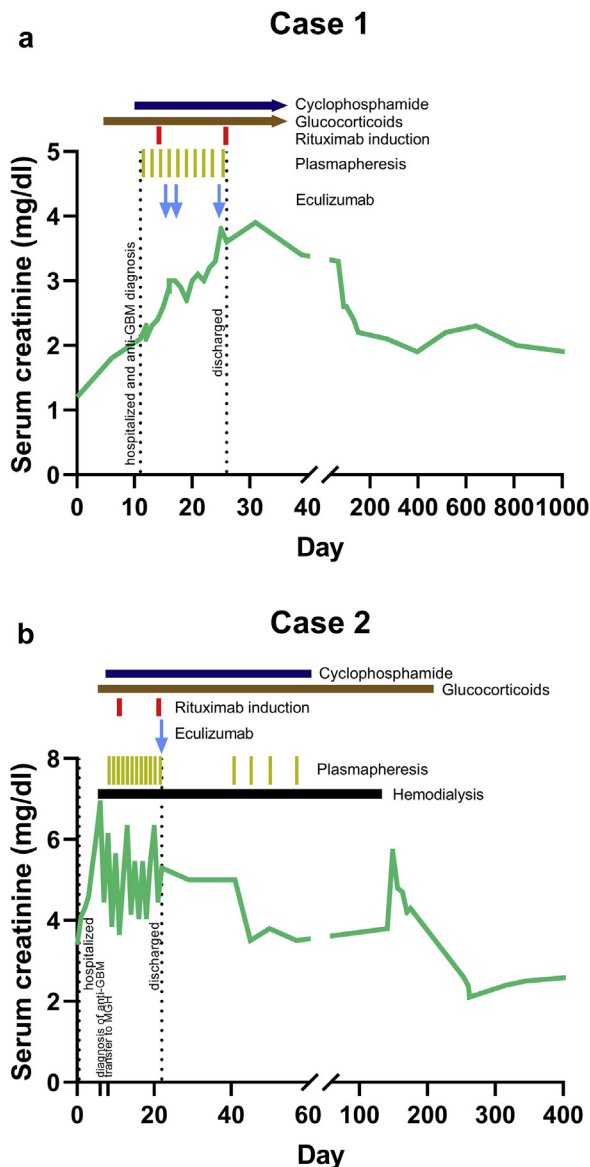
Figure 1. Clinical course of patients treated with eculizumab. (a) In case 1, the patient is treated with plasmapheresis (gold bars), 2 induction doses of rituximab 1000 mg i.v. (red bars), a 6-month terminal prednisone taper (brown horizontal arrow), and oral cyclophosphamide dosed at 2.5 mg/kg per day for 1 week, then 1.5 mg/kg per day for 7 weeks, with doses adjusted for renal function (received a cumulative dose of 3.8 g) (blue horizontal arrow). Three doses of eculizumab at 300 mg, 600 mg, and 900 mg, were given following the fifth, sixth, and tenth plasmapheresis sessions, respectively (light blue vertical arrow). The patient's serum creatinine reached a zenith of 3.9 mg/dl, and following treatment his SCr improved to 1.9 mg/dl. (b) In case 2, the patient underwent plasmapheresis (gold bars) followed by additional sessions for a rising anti-GBM titer, 2 induction doses of rituximab 1000 mg i.v. (red bars), a 6-month terminal prednisone taper (brown horizontal bar), and oral cyclophosphamide dosed at 2.5 mg/kg per day for 1 week, then 1.5 mg/kg per day for 7 weeks, with doses adjusted for renal function (received a cumulative dose of 3.15 g) (blue horizontal bar). One dose of eculizumab 900 mg i.v. was given (light blue vertical arrow) following the 12th plasmapheresis session. The patient's SCr reached a zenith 6.9 mg/dl before initiating hemodialysis (black horizontal bar). At 4 months following hospital discharge, hemodialysis was discontinued, and her SCr improved to 2.6 mg/dl.

Case 2

A 58-year-old woman presented with fatigue, dyspnea, anorexia, and dysgeusia progressing over a 2-week period. Her SCr was 3.4 mg/dl (unknown baseline), and urinalysis was significant for blood (3+) and protein (2+). Urine sediment showed numerous red blood cell casts. On hospital day 3, a kidney biopsy was performed. By hospital day 6, a diagnosis of anti-GBM disease was made after the biopsy specimen revealed necrotizing glomerulonephritis with linear IgG deposition along the glomerular capillary walls (Supplementary Figure S2). Crescents were present in 70% of viable glomeruli. Testing for anti-GBM was positive at 636 RU/ml (negative < 20 RU/ml) and testing for ANCA was negative. In addition, on hospital day 6, her SCr reached a zenith of 6.9 mg/dl, hemodialysis was initiated, and arrangements were initiated to transfer the patient to our institution for plasmapheresis. Moreover, glucocorticoids and cyclophosphamide were initiated at the outside hospital on hospital day 6. Her treatment included methylprednisolone 1000 mg i.v. daily for 3 days followed by a prednisone taper and oral cyclophosphamide initially dosed at 2.5 mg/kg per day with adjustments made for renal function (Figure 1b). She arrived at our institution on hospital day 8 and received daily plasmapheresis and rituximab. Additional evaluation of the kidney biopsy specimen demonstrated positive staining for C5b-9 in a distinctly granular pattern in the glomeruli (Figure 2a). It also demonstrated positive staining for C4d, C3, C3d, and C5b-9 in the glomeruli, tubular basement membrane, and vessels (Figures 2; Supplementary Figure S3, S4). The patient received eculizumab 900 mg i.v. after the 12th plasmapheresis treatment prior to discharge. Four months after discharge, dialysis was discontinued. At last follow-up 1 year after initial presentation, her SCr was at her new baseline of 2.6 mg/dl with absent hematuria, and her anti-GBM antibody titer was consistently negative (Supplementary Figure S1).

DISCUSSION

Historically, deposition of C3 along the GBM has been frequently observed in biopsy specimens of patients with anti-GBM disease, which theoretically suggests a pathogenic role of complement.[1] To date, a limited number of experiments evaluating this theory have been published. In 1984, Groggel et al. demonstrated that rabbits deficient in C6 had less proteinuria and a smaller rise in serum creatinine in an anti–GBM disease model, which suggested that the membrane attack complex (MAC; also known as C5b-9) contributes to renal injury.[2] In 1997, Sheerin et al. demonstrated that mice deficient in C3 or C4 had less neutrophilic infiltration, less
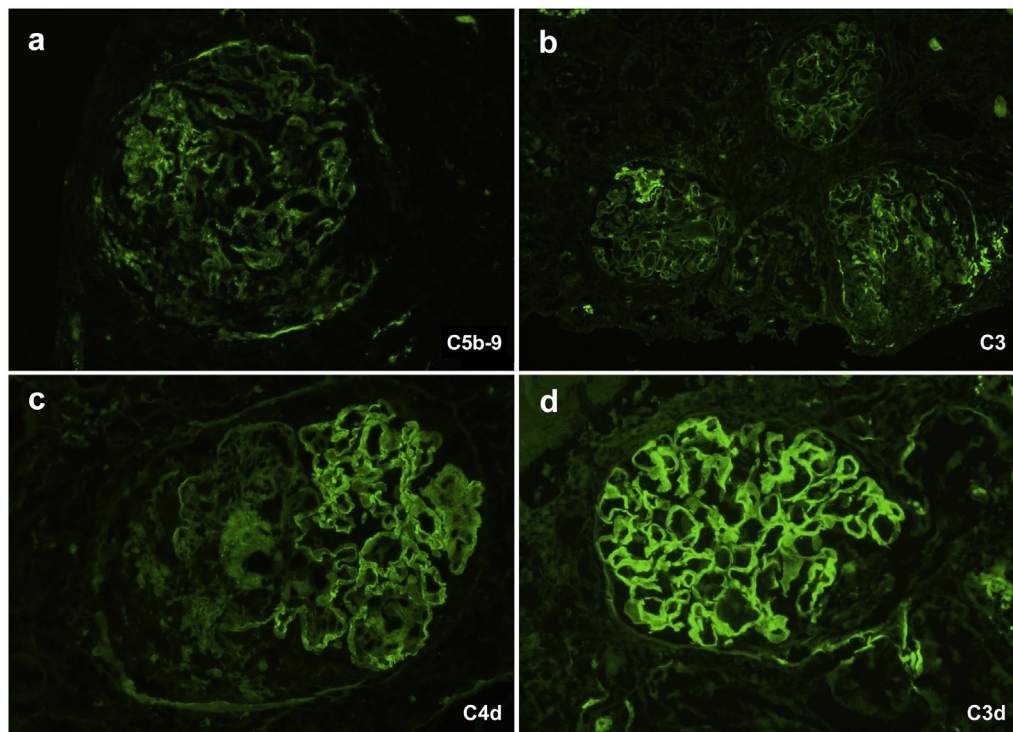
Figure 2. Kidney biopsy findings of case 2 patient: immunofluorescent photomicrograph of glomeruli. (a) Granular C5b-9 (membrane attack complex) deposition is detected along the glomerular capillary wall. There was also detection of (b) C3, (c) C4d, and (d) C3d along the glomerular capillary loops. b, Original magnification ×20; a, c, and d, original magnification ×40.

glomerular capillary thrombosis, and less proteinuria compared to wild-type mice in an anti-GBM disease model.[3] In 2013, Ma *et al.* found elevated serum and urinary levels of C5b-9 and C5a in humans with anti-GBM disease.[4] They also observed an association between the serum C5b-9 level and development of renal failure. Finally, in 2014, Ma *et al.* found that C1q, factor B, properidin, C3d, C4d, and C5b-9 were detected along the GBM in all the glomeruli in 10 patients with anti-GBM disease (5). These findings suggest that complement activation might play a significant role in renal damage in anti-GBM disease (Table 1).

Our patient's kidney biopsy specimen from case 2 revealed extensive C5b-9 deposition in the glomerular capillary walls, along with detection of C3, C3d, and C4d. High circulating levels of and positive biopsy staining for C5b-9 suggest activation of the terminal complement cascade and offer a mechanistic rationale for therapeutic complement inhibition. In the study by Ma *et al.*, C5b-9 was detected clearly in a linear pattern along the

glomerular capillary wall in 29 glomeruli (100%) of all 10 consecutive patients with anti-GBM disease.[5] In contrast, no deposits of C5b-9 were found in renal biopsy specimens from patients with minimal change disease (n = 5), a disease in which complement activation is not thought to be involved, nor was it detected in normal control renal tissue (obtained from the normal part of a nephrectomized kidney due to renal carcinoma). The investigators also observed stronger-intensity C5b-9 staining in glomeruli with crescent formation compared with the glomeruli without crescent formation. Although further study is required to validate the role and interpretation of C5b-9 staining in anti-GBM disease, C5b-9 staining has been studied in other diseases causing glomerulonephritis. For example, C5b-9 staining on kidney biopsy specimens can be found in nearly all patients with proliferative lupus nephritis.[6] Although the intensity of staining did not significantly differ between active and chronic forms, posttreatment biopsy specimens did show a reduction in C5b-9 staining intensity. A similar reduction in C5b-9

Table 1. Teaching points

Teaching points
Current therapeutic strategies for **anti–glomerular basement membrane** (anti-GBM) disease include daily plasmapheresis, glucocorticoids, cyclophosphamide, and rituximab.
Rapid initiation of immunosuppressive therapy is paramount to prevent irreversible kidney damage, dialysis dependence, and death.
Emerging data suggest that complement activation might play a significant role in renal damage in anti-GBM disease.
Complement activation can be triggered by anti-GBM antibody binding, which can generate and amplify the inflammatory response, including the recruitment and activation of leukocytes.
The use of terminal complement blockade with eculizumab, an anti-C5 monoclonal antibody, has the potential to be an effective novel therapy for anti-GBM disease. Its therapeutic role requires further investigation.

staining intensity after treatment has been reported in dense deposit disease.[7] In our patient, given the clinical and serologic improvement, a repeat biopsy posttreatment was deferred.

In addition to complement deposition in the glomerular capillary walls, we detected staining for C5b-9, C3, C3d, and C4d in the tubular basement membrane and vessels outside the glomerulus (Supplementary Figures S3, S4). Given that the non-collagenous domain of the α3 chain of type IV collagen, also known as the Goodpasture antigen, is largely localized to glomerular and alveolar basement membranes, possible mechanisms to explain complement activation outside of the glomerulus include the presence and recognition of the target autoantigen, cross-reactivity with another antigen, or nonspecific staining from nearby complement activation. Future biopsy studies with controls would be informative.

There is growing evidence that complement blockade in ANCA-associated vasculitis (AAV) may be effective,[8,S1] and it is possible that the potential therapeutic response that the patient in case 1 had to eculizumab was to the component of his presentation due to AAV. Moreover, the patient's initial symptomatology and time course can be typical for AAV as the predominant driver of disease. However, the patient had an unequivocally positive anti-GBM titer and developed relentlessly worsening kidney function despite high-dose glucocorticoids, plasmapheresis, rituximab, and cyclophosphamide, which can be seen with the aggressive nature of many cases of anti-GBM disease. Despite patient 1 initially possessing good prognostic features, the impetus for using eculizumab as rescue therapy related to the rate of rise in serum creatinine and the rate of decline in urine output without an alternative explanation while the patient was already receiving full conventional therapy.

The overall and renal prognosis of anti-GBM disease depends largely on how quickly immunosuppressive therapy is initiated. In a retrospective observational study, in patients presenting with a SCr ≥5.7 mg/dl but not requiring dialysis within 3 days of admission (n = 13), the patient and renal survival were 83% and 82% at 1 year, respectively.[9] In patients who required dialysis within 3 days of admission (n = 39), the patient and renal survival were 65% and 8% at 1 year, respectively. Finally, all patients who required dialysis within 3 days and had 100% crescents on renal biopsy specimens remained dialysis dependent. In our series, patient 2's presenting SCr was 3.4 mg/dl; however, immunosuppressive therapy was not initiated until the SCr was 6.9 mg/dl. She required dialysis 6 days after admission, and her biopsy specimen revealed 70% crescents. Furthermore, she received 1 dose of eculizumab 16 days after a

diagnosis was established (22 days after admission). It is possible that the limited and delayed initiation of eculizumab blunted any potential efficacy due to the onset of irreversible kidney damage. Given the patient's ongoing dialysis dependence, eculizumab was used here as an immediate-acting anti-inflammatory agent at the level of the end organ to further bridge the patient until other therapies addressing more upstream pathogenic autoreactive B cells, plasma cells, and circulating autoantibodies took greater effect. It is difficult to say whether our patient's clinical outcome of dialysis independence would have been expected without eculizumab. Future studies investigating anticomplement therapy should include earlier initiation and potentially more prolonged therapy courses. Moreover, no adverse events directly attributable to complement inhibition were observed in our patients, both of whom received vaccinations for meningococcus serogroups ACWY and B, as well as chemoprophylaxis with penicillin. However, a control group is necessary to determine the efficacy and safety of eculizumab in anti-GBM disease. Our observations serve as grounds for further scientific investigation for targeting complement in a disease for which treatment advancements are needed.

DISCLOSURES

All the authors declared no competing interests.

PATIENT CONSENT

The authors declare that they have obtained consent from the patients discussed in the report.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Anti-glomerular basement membrane (anti-GBM) antibody titers over disease course. (A) In case 1, the patient's anti-GBM antibody titer became negative (<20 RU/ml) at cessation of plasmapheresis. (B) In case 2, the patient's anti-GBM antibody titer rebounded after discharge from the hospital, warranting 4 additional plasmapheresis sessions, after which the patient's titer became consistently negative. Testing was performed by western blot analysis. Day 0 is the day of initial presentation. GBM, glomerular basement membrane; MGH, Massachusetts General Hospital; RU, relative units.

Figure S2. Kidney biopsy findings of case 2 patient: light microscopy and IgG immunofluorescence. (A) Periodic

acid−Schiff stain of a core needle-biopsy specimen from the case 2 patient. Glomeruli are shown with cellular crescents (black asterisk) and necrosis (original magnification ×20). (B) Linear IgG staining along the glomerular capillary wall on immunofluorescence.

Figure S3. Kidney biopsy findings of case 2 patient: immunofluorescent photomicrography of tubular basement membranes. Detection of granular deposition of (A) C5b-9, (B) C4d, and (C) C3d along the tubular basement membranes. A, C, original magnification ×20; B, original magnification ×40.

Figure S4. Kidney biopsy findings of case 2 patient: immunofluorescent photomicrography of vessels. Detection of granular deposition of (A) C5b-9, (B) C3, (C) C4d, and (D) C3d along the vessel wall. A, D, original magnification ×20; B, C, original magnification ×40.

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