Binding Truths: Seronegative Anti-Glomerular Basement Membrane Disease Mediated by IgM Anti-Glomerular Basement Membrane Antibodies

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INTRODUCTION

Anti-glomerular basement membrane (anti-GBM) disease presents with rapidly progressive glomerulonephritis and frequently with associated alveolar hemorrhage. It is typically characterized by diffuse crescentic glomerular inflammation and linear deposition of immunoglobulin along the GBM, which in most cases, is due to IgG autoantibodies directed against the noncollagenous domain of the α3 chain of type IV collagen (α3[IV]NC1).[1]

Early serologic diagnosis allows for prompt initiation of plasma exchange and immunotherapy, which is beneficial because outcomes are dependent on the severity of renal involvement. However, conventional assays do not detect IgA or IgM antibodies or those directed against other target antigens, including α5(IV) found in some Alport's disease patients following renal transplantation.[2] This makes the diagnosis and treatment of seronegative anti-GBM disease challenging.

We have previously described a case of anti-GBM disease mediated by IgA anti-GBM antibodies against the collagenous domain of α1(IV)[3] undetected by standard serologic tests, and suggested a method of detection and monitoring that could be used in the right clinical context. In this report, we describe 2 cases of anti-GBM disease mediated by IgM antibodies and have adopted the same method to determine the pattern of IgM deposition along the GBM using patient serum during acute and convalescent disease. This highlights the need to adapt assays to be used in the right clinical context to enable rare but important variants in this condition to be properly characterized and monitored.

CASE PRESENTATION

Patient 1

A 52-year-old female presented oligoanuric with nephrotic range proteinuria and microscopic hematuria, 1 month history of malaise and acute kidney injury requiring hemofiltration. She had a past medical history of Crohn's disease and colorectal cancer resected with curative intent 16 years before her presentation with nephritis.

Her blood cultures grew 2 organisms, namely *Ochrobactrum anthropi* and *Staphylococcus epidermidis*. She had pancytopenia and low complement levels. Her autoantibody serology, including antinuclear antibody, antineutrophil cytoplasmic antibody, IgG anti-GBM, and HIV and hepatitis screen were negative. Computed tomography ruled out significant alveolar hemorrhage.

Immunoglobulin levels were within normal range, and she had low serum C3 and C4 levels. Her serum immunofixation revealed type 3 polyclonal cryoglobulin. No Bence Jones proteins were detected in the urine.

Renal biopsy demonstrated significant chronic damage in the tubulointerstitium occupying approximately 10% of the cortical area and 50% of the subcapsular core. Six out of the 12 glomeruli were globally sclerosed and 4 contained large cellular crescents, with fibrin deposition, blood, and inflammatory cells. There was no deposition of IgG or IgA in glomeruli but there was linear deposition of complement C3 and IgM, along the GBM (Figure 1).

She received plasma exchange and methylprednisolone followed by a weaning regime of oral prednisolone
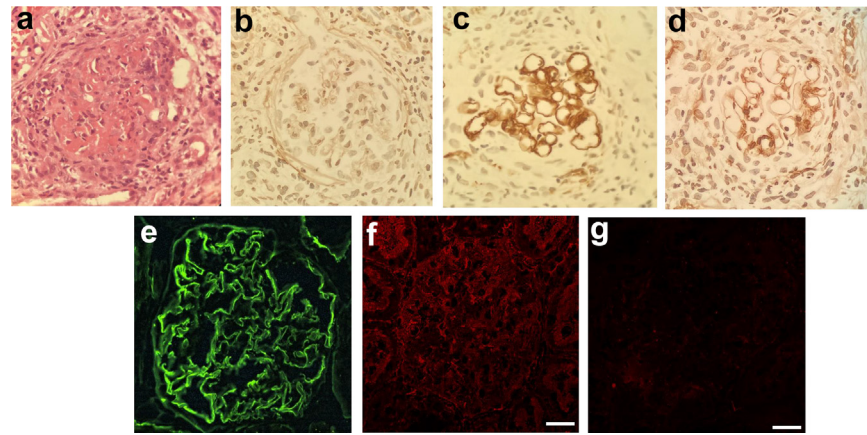
Figure 1. Patient 1 renal biopsy tissue stained with (a) hematoxylin and eosin showing a large circumferential cellular crescent, and stained by immunohistochemistry for (b) IgG, (c) IgM, and (d) C3. Indirect immunofluorescence staining of primate kidney sections (Euroimmun, product no FB 1250-1010) incubated overnight at 4 °C with (e) control patient serum with IgG antibodies against the non-collagenous domain of the α3 chain of type IV collagen, (f) patient acute, and (g) patient convalescent serum. Patient's and control sera (diluted 1:10) antibody binding was visualized with Alexa Fluor 647- labelled secondary antibodies (diluted 1:1000) to human IgM (diluted 1:250) (f, g) or IgG (diluted 1:500) (e). The scale bar represents 30 μm.

and 2 doses of rituximab. She remained oligoanuric and was eventually established on hemodialysis 1 month after treatment.

In a modified indirect immunofluorescence method we previously described, we used patient serum to stain primate kidney sections, which confirmed prominent linear IgM binding to the GBMs, and the tubules, and Bowman's capsule. This binding may imply a different antigen to the classical a3(IV) NC1 as we have previously shown with a case of IgA anti-GBM that can be further identified with enzyme-linked immunosorbent assays and western blotting.[3] Reassuringly, there was no such staining with convalescent serum obtained 3 months after treatment initiation, allowing the patient to be placed on the transplant waiting list (Figure 1).

Patient 2

A 78-year-old female presented with 1 month history of malaise and oliguria requiring hemodialysis. She had no significant past medical history.

Her autoantibody serology and serum electrophoresis were negative, and immunoglobulins were within normal range. She had a low serum C3 level with C4 within normal range. Her echocardiogram showed no vegetations.

Her renal biopsy showed significant damage with 88% chronicity index. Most of the glomeruli were sclerosed but contained cellular crescents. There was linear deposition of complement and IgM along the GBM, in the absence of IgA and IgG deposition (Figure 2).

She was treated with methylprednisolone and cyclophosphamide that was discontinued early because

of infection risk and ongoing need for renal replacement therapy. She remained hemodialysis dependent until she died 2 years later.

DISCUSSION

We describe 2 patients with end-stage renal failure secondary to anti-IgM GBM disease, as defined by the presence of crescentic glomerulonephritis in the renal biopsies with linear IgM and complement deposition along the glomerular capillaries. In the first patient, a type 3 cryoglobulin was detected on serum immunofixation.

Non-IgG anti-GBM disease is extremely uncommon. In over 50 patients that presented with anti-GBM disease in our renal unit over the last 3 decades, we have already reported a single case of anti-IgA-mediated disease.[3] These are the only 2 cases of IgM-mediated disease we have encountered. A previous publication by Nasr *et al.*[4] of 20 cases of "atypical anti-GBM disease" have been reported, characterized by bright, linear GBM immunoglobulin deposition in patients who lacked anti-GBM antibodies by conventional testing and had a relatively benign course. Most of these cases had IgG anti-GBM binding, and only 2 cases were identified as having linear IgM GBM-deposition without concomitant C3 deposition.

Our 2 cases differ from the cases described in the Nasr series in clinical and histologic severity because both our patients developed end-stage renal failure requiring hemodialysis, with low serum C3, and both had glomerular crescents and C3 deposition on their biopsies, demonstrating pathologic anti-GBM activity.

Anti-GBM disease may be regarded as a "conformeropathy" where the quaternary structure of the
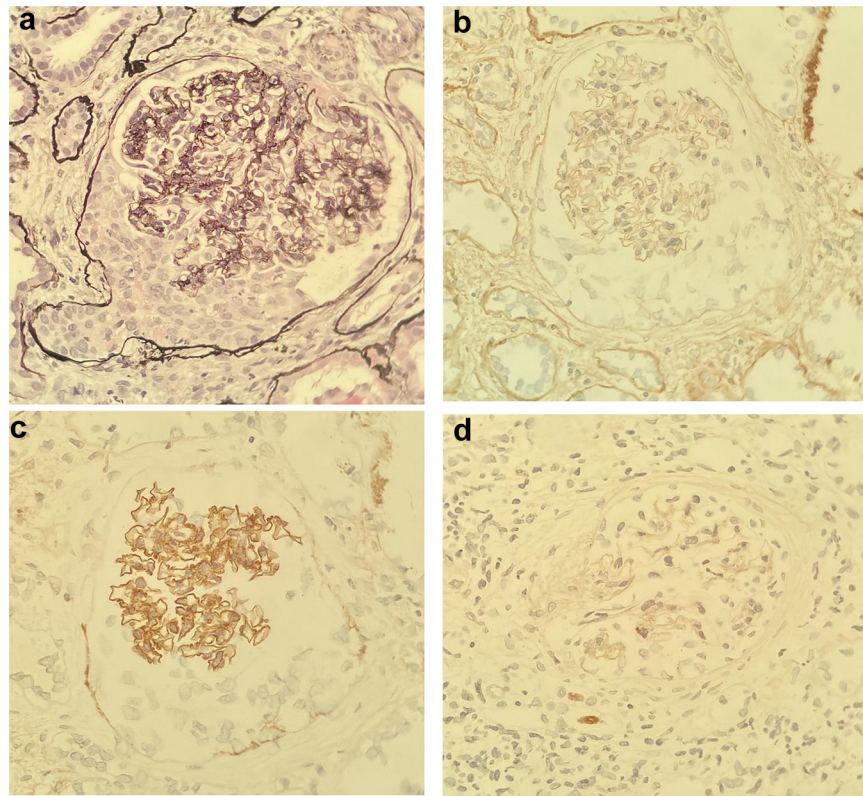
Figure 2. Patient 2 renal biopsy tissue stained with (a) hematoxylin and eosin showing a large circumferential cellular crescent, and stained by immunohistochemistry for (b) IgG, (c) IgM, and (d) C3.

α3(IV)NC1 hexamer that constitutes the GBM undergoes a conformational change, resulting in the modification or exposure of "hidden" epitopes that may elicit a pathogenic autoantibody.[5] This may account for the association of anti-GBM disease with etiologic factors that may disrupt the GBM or other renal pathologies[S1–S3] that can often precede anti-GBM disease.

The mechanism of glomerular IgM deposition and its clinical significance remains controversial. Glomerular IgM deposition has been reported in a wide range of glomerular diseases.[S4–S7] Natural IgM antibody has been reported to have a beneficial role in binding self-epitopes generated during ischemia,[6] and aiding in the removal of injured tissue.[7] Therefore, passive trapping of the IgM within areas of sclerosis might explain the glomerular deposition of IgM in this broad array of glomerular diseases. However, passive trapping would not explain the often seen codeposition of complement, the absence of other immunoglobulin deposition such as IgG and the severe presentation with end-stage renal failure requiring renal replacement therapy that we have described in these 2 cases.

Several observations support the hypothesis that IgM specifically binds epitopes in injured or stressed glomeruli that exacerbates disease. The presence of IgM in patients with nephrotic syndrome is associated with

steroid unresponsiveness and is predictive of progression to end-stage renal disease.[S5] In addition, in patients with lupus nephritis, glomerular IgM contributes independently to glomerular C3 deposition.[8] In a murine model of complement-mediated glomerular injury, IgM was deposited linearly along the GBM and animals unable to produce IgM developed milder disease histologically.[9] In addition to supporting the concept that IgM binding is a specific process, the authors identified 2 IgM clones that deposit specifically along the GBM of animals with glomerulonephritis but do not bind within the capillaries of wild-type mice.

In conclusion, we hypothesize that in the presence of damage to the glomerular membrane and the exposure of "hidden epitopes," IgM can also contribute to

Teaching points

- Conventional anti-GBM assays used in clinical practice would not detect IgA or IgM antibodies or those directed against other target antigens such α5(IV) found in some Alport disease patients following renal transplantation.

- Natural IgM can have a beneficial role in clearing damaged and apoptotic cells and protecting the individual from infection. However, linear IgM glomerular deposition in the presence of crescentic glomerulonephritis can be pathologic requiring immunosuppressive treatment.

- If the clinical presentation and histopathological findings are suggestive of anti-GMB disease in the absence of positive serology results, alternative laboratory tests such as immunoblotting and indirect immunofluorescence can be used to confirm the diagnosis.

GBM, glomerular basement membrane.

tissue injury through activation of the complement system and autoantibody production against the exposed epitope with subsequent generation of auto-immunity and tissue inflammation.

DISCLOSURE

All the authors declare no competing interests.

PATIENT CONSENT

Patient consent has been obtained.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplemental References.

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