EDITORIAL COMMENT

Atypical anti-glomerular basement membrane disease: lessons learned

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Abstract

Anti-glomerular basement membrane (GBM) disease usually pursues a self-limited course, at least from the immunological perspective. In addition, circulating antibodies to cryptic, conformational epitopes within the NC1 domain of the alpha 3 chain of Type IV Collagen are commonly found at the zenith of the clinical disease. However, exceptions to these general rules do occur, as exemplified by two remarkable cases reported in this issue of the Clinical Kidney Journal. The possible explanations for and the lessons learned from these uncommon occurrences are discussed in this short commentary.

Anti-glomerular basement membrane (GBM) antibody disease is an uncommon but very well understood autoimmune disease, first delineated by Lerner, Glassock and Dixon in 1967 [1]. It has been known for over two decades that the predominant pathogenic autoantibody is of the IgG isotype and is directed to epitopes on the non-collagenous (NC) domain of Type IV collagen, chiefly, but not exclusively, in a peptide sequence of the alpha 3 chain [2, 3]. The two major epitopes (Ea and Eb) are cryptic and conformational, residing in the hexamer of the alpha 3, 4, 5 chains of Type IV collagen [2]. Dissolution of sulfilimine bonds and dissociation of the hexamer are required for binding of anti-GBM autoantibodies [4]. The initiating mechanism(s) for anti-GBM autoantibody formation remain obscure, but recent data suggest that 'autoantigen complementarity' might be involved [5].

A large number of assays for such autoantibodies have been developed and described, some having commercial applications, while others are primarily of research interest [6–11]. These assays utilize a variety of basement membrane substrates and detection technology [e.g. indirect immunofluorescence (IIF), radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), chemiluminescence and western blot (WB)] [6, 8–11]. These assays have variable but generally high specificity (94–100%) and generally high sensitivity (95–100%), with highest sensitivity for the WB assay and a chemiluminescent assay involving human recombinant GBM antigen and lowest for the direct immunofluorescence (IF) microscopy assays [8–11], with lower specificity for assays using extractive rather than human recombinant GBM antigens [9]. A highly sensitive bio-sensor assay has also recently been described, but is generally not available for clinical use [6]. Anti-GBM antibody undetectable by the ELISA methodology using recombinant GBM antigens might be caused by failure to detect cryptic or conformational epitopes, distinct from the Ea or Eb epitopes. Such cases may be detected by IIF [8]. ELISA assays are also most specific and sensitive for IgG1 and IgG3 antibodies and may be falsely negative when IgG4 or IgA anti-GBM antibodies are involved in disease pathogenesis [7, 12].

Furthermore, it is well known that the anti-GBM antibodies in human disease are intrinsically heterogeneous with respect to their reactivity to GBM constituents, but only some anti-GBM antibodies are pathogenic [2, 8, 13, 14]. The origin of this heterogeneity is uncertain, but might be due to secondary release of altered or cryptic autoantigens from damaged tissue or due to intra- or intermolecular epitope spreading in evolving disease [15]. Importantly, the assays for anti-GBM antibody utilize a variety of substrates: frozen whole tissue in IIF, isolated purified bovine or human GBM antigens, or recombinant human alpha 3 chain of the NC domain of Type IV collagen. These considerations of assay variability assume clinical importance in evaluation of possible serum-based 'false negative' tests (e.g. negative tests in the face of linear IgG deposits by IF in the glomeruli showing a crescentic injury pattern by light microscopy) or 'false positive' tests (positive tests in the face of absence of linear IgG deposits by

immunofluorescence). For the commonly employed assays (ELISA), using human or bovine substrates, false-positive results are quite uncommon (around <1%), while false-negative results are more numerous (around 5% or less). Thus, the origins of the false-negative results are potentially quite varied. To summarize, they include: (i) the intrinsic sensitivity of the assay, especially for low-affinity antibodies [9]; (ii) anti-GBM antibody isotypes or subclasses (IgA or IgG4) not easily detected in ELISA or RIA [7, 12]; (iii) antibody disappearance from the circulation before the resolution of disease; (iv) an 'immunological sink' where high-affinity antibodies are quickly removed from the circulation, leaving behind only low levels of low-affinity antibodies that escape detection; (v) an effect of the substrate—lack of epitopes reacting with the antibodies highly specific for human GBM, not shared by non-human (non-primate) antigens; and (vi) involvement of T-cells or other mediators (such as complement or cytokines), rather than antibodies *per se* in the pathogenesis of tissue injury [16].

Many examples of putative 'antibody negative' atypical anti-GBM disease have been described [6, 7, 17–20]. These largely anecdotal reports have led to the suggestion that no single test can replace the accuracy of a good quality kidney biopsy and a nephrologist's clinical acumen in diagnosing Goodpasture's disease (now known generally as anti-GBM disease) [18]. Of course, a positive anti-GBM assay performed in a respected laboratory has a very high predictive value, in a clinically compatible scenario, approaching 99.5%.

Another very common feature of anti-GBM disease is its 'one-shot' characteristic [21–24]. Typically, the disease begins abruptly, often with a 'viral-like prodrome', and severe but potentially reversible renal injury [usually crescentic glomerulonephritis (GN)] evolves rapidly, with or without pulmonary hemorrhage [21]. It then naturally subsides, often over a protracted period without specific treatment, leaving behind a variable degree of lasting glomerular injury, depending on the magnitude of the initial injury, the timeliness and aggressiveness of treatment, most often associated with the disappearance of circulating anti-GBM antibodies [21, 22]. Atypical forms of presumed anti-GBM disease not associated with crescentic GN have been described [25]. Many of these patients (≈50%) have monoclonal disorders and none has circulating anti-GBM antibodies, at least not those directed to the typical NC1 domain of alpha 3 Type IV collagen [25]. In 'classic' anti-GBM disease there is a low probability of recurrence or relapse, even in the absence of continued immunosuppressive therapy. In the large series of cases from Hammersmith Hospital in the UK, only 2 of 71 (3%) patients had a subsequent relapse after initial treatment [23]. Thus, conventional management entails only rather short courses (about 3–6 months in duration) of intensive therapy, including plasma exchange (PLEX; daily for 14 days or until anti-GBM antibodies are undetectable), oral cyclophosphamide (2–3 mg/kg/day for 2–3 months) and oral steroids (initially 1 mg/kg/day, slowly tapered over about 6 months), if the patient is not dialysis-dependent [22, 23]. Clearly, this is the 'usual' course of *classic* anti-GBM disease, with prominent renal involvement, but like 'antibody negative' anti-GBM disease, exceptions to this general rule have been sparsely documented, usually as case reports or anecdotes of relapsing disease [26]. The precise mechanisms underlying this uncommon recurring and relapsing form of anti-GBM disease are obscure, but T-cell-mediated modulation of the immune response has been suggested, based on experimental studies of animal models of the disease [16].

Both of these phenomena are described in two exceptional case reports published in this issue of the *Clinical Kidney Journal* by Liu *et al.* [27] and by Gu *et al.* [28]. Liu *et al.* [27] describe a case of a 33-year-old woman, a heavy smoker, who developed crescentic GN with linear deposition of IgG (not characterized for subclass) and negative anti-GBM antibodies in an ELISA assay (substrate not specified). No pulmonary hemorrhage (PH) was observed initially. The patient subsequently experienced two relapses, one 5 years after the initial episode, this time with PH and positive anti-GBM. She was treated with PLEX, steroids and cyclophosphamide, the latter for 32 months—a period greatly exceeding usual practice. A second relapse occurred 8 years later, again with PH, but with negative anti-GBM antibodies. She improved with treatment and no further relapses have occurred despite continued cigarette smoking.

Gu *et al.* [28] describe a case of a 41-year-old woman with PH and crescentic GN with linear IgG (IgG 2 dominant) and positive anti-GBM (ELISA assay using bovine alpha 3 Type IV collagen substrate). She recovered with standard therapy, but was left with residual chronic kidney disease [estimated glomerular filtration rate (eGFR) = 32 mL/min/1.73 m^2]. A relapse of PH alone without any significant change in renal status or positive anti-GBM antibody developed 4 years later and a repeat renal biopsy showed 'inactive' disease, but persistence of linear deposition of IgG along the glomerular capillary walls. The PH responded to therapy (including PLEX) and she was given azathioprine 'prophylaxis'. However, PH recurred again 2 years later and a renal biopsy again showed no 'activity', but linear IgG deposition persisted. She improved with treatment but a third relapse of PH subsequently developed, this time with worsening of renal function (eGFR = 6 mL/min/1.73 m^2), requiring dialysis treatment. Standard therapy again improved the PH, but she was left dialysis-dependent. Like the case of Liu *et al.* [27], she was a lifetime smoker and also had continual exposure to paint and solvents as well as to cigarette smoke.

Taken together, these two cases provide an important lesson that a monophasic illness is not inevitable in anti-GBM disease, and detection of anti-GBM antibodies can be quite variable (depending on the assay used). These observations extend the phenotype of antic-GBM disease. Neither case was associated with coexisting anti-neutrophil cytoplasmic autoantibodies (ANCAs), an important cause of a relapsing course in 20–30% of patients with anti-GBM disease [29]. A common feature in these two cases is the continuing exposure to pulmonary irritants and prominent manifestations of PH with relapses. This might explain the repeated bouts of PH in the presence of a low (and undetectable by conventional assays) levels of possibly low-affinity anti-GBM antibodies. These antibodies might have been detected by WB or bio-sensor-based assays, which were not performed. The continued presence of linear deposits of IgG in the case of Gu *et al.* [28] is compatible with this hypothesis. High-affinity anti-GBM antibodies may also be 'quenched' by *in vivo* immunoadsorption onto available tissue sites (the 'immunological sink' hypothesis), leaving only low-affinity antibody for detection in the circulation, and increasing the likelihood for a 'false negative' result in ELISA anti-GBM antibody assays. WB or chemiluminescence assays are preferred when the ELISA assays are inextricably negative; however, these assays may be of limited availability. It is of some interest that in the original description of anti-GBM antibody disease in 1967 [1], antibody levels increased dramatically following bilateral nephrectomy—indirect evidence supporting the 'immunological sink' hypothesis. The observation of a response of PH to PLEX, cyclophosphamide and steroid therapy despite the absence of detectable anti-GBM antibodies implies that removal of some substance or replacement of a missing factor was involved. While this may have been low-affinity anti-GBM antibody, other factors might also be involved, such as complement components or cytokines. But this is pure speculation.

In any case, while many questions cannot be answered, these two exceptional cases re-emphasize the broad spectrum of anti-GBM disease and illustrate the limitations of presently available (unstandardized) assays for anti-GBM antibody. The nature of these cases are quite insufficient to recommend altering the current clinical practice guidelines for management of newly diagnosed anti-GBM disease, which emphasize/advocates short courses of active immunosuppressive therapy without the need for long-term prophylaxis [30]. They also highlight the need for early renal biopsy and direct IF microscopy for diagnosis of anti-GBM disease in clinically suspected cases even when anti-GBM assays on serum are 'negative' (by commercially available assays). An IgG4- or IgA-mediated anti-GBM disease should be suspected also when conventional IgG-specific assays for circulating anti-GBM antibodies are negative [7, 12]. One cannot be sure if PLEX is always indicated in PH with tissue deposits of IgG in a linear pattern despite 'negative' serum anti-GBM antibodies, but one should err on the side of pursuing PLEX if the PH is severe and life-threatening, in my opinion. The option of bilateral nephrectomy has been largely abandoned as a maneuver to prevent recurrent anti-GBM disease in renal allografts [31], but anecdotes of improvement in the activity of native disease and refractory, persistently high anti-GBM antibody levels following bilateral nephrectomy have been reported [32]. The evidence that the diseased kidneys somehow are involved, in provoking continued autoantibody production resistant to immunosuppressive therapy perhaps by release of altered GBM antigens, is weak and unsubstantiated. Finally, every effort should be made to remove subjects from exposure to pulmonary irritants of any kind in patients with a diagnosis of anti-GBM disease, regardless of the presence or absence of detectable circulating anti-GBM antibodies. All patients with anti-GBM disease also deserve regular and close follow-up, even for years after the initial episode, even though only a very few will pursue a course revealed by these two highly instructive cases.

Conflict of interest statement

None declared.

(See related article by Liu *et al*. Multiple recurrences of anti-glomerular basement membrane disease with variable antibody detection: can the laboratory be trusted? *Clin Kidney J* (2016) 9: 657–660 and by Gu *et al*. Frequently relapsing anti-glomerular basement membrane antibody disease with changing clinical phenotype and antibody characteristics over time. *Clin Kidney J* (2016) 9: 661–664.)

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