The clinical and immunological features of patients with combined anti-glomerular basement membrane disease and membranous nephropathy

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The association of anti-glomerular basement membrane (GBM) disease, also known as Goodpasture's disease, with membranous nephropathy (MN) has been well documented. However, little is known about the clinical and immunological features of patients with such a combination. This study was designed to investigate the clinical and immunological features of anti-GBM patients with MN and to provide insight into the pathogenesis of this rare entity. Eight patients with combined anti-GBM disease and MN were found to have significantly lower levels of serum creatinine, a significantly lower proportion of oliguria/anuria, and significantly better renal outcomes compared with 30 patients with classical anti-GBM disease. Antibody levels against the E_B conformational epitope of anti-α3(IV)NC1 were significantly lower in these patients, as was their levels of anti-α3(IV)NC1 immunoglobulin G1 (IgG1) and IgG3. Serum antibodies against the M-type phospholipase A_2 receptor were undetectable in anti-GBM patients with MN but presented in 13 of the 20 patients with primary MN. Thus, patients with combined anti-GBM disease and MN have distinct clinical features and a different immunological profile of MN.

Kidney International (2014) **85,** 945–952; doi:10.1038/ki.2013.364; published online 18 September 2013

KEYWORDS: anti-GBM antibodies; antigen; epitope; IgG subclasses; membranous nephropathy; M-type phospholipase A_2 receptor

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Received 13 October 2012; revised 8 July 2013; accepted 1 August 2013; published online 18 September 2013

Anti-glomerular basement membrane (GBM) disease, also known as Goodpasture's disease, is an autoimmune disorder characterized by the production of anti-GBM autoantibodies, rapidly progressive glomerulonephritis with crescent formation, and high risks for alveolar hemorrhage. The pathogenic role of anti-GBM antibodies has been fully demonstrated.[1,2] These antibodies primarily target the noncollagenous domain 1 of α3 chain of type IV collagen (α3(IV)NC1)[3] and, more specifically, bind to two major conformational epitopes named E_A and E_B, which are normally sequestrated in the quaternary structure of GBM.[4–7]

Membranous nephropathy (MN), a common cause of nephrotic syndrome in adults, is characterized by the presence of subepithelial immune complexes, subsequent complement activation, resultant basement membrane damage, and proteinuria. Crescents are rare in MN and their presence often suggests another underlying disease process, including anti-GBM disease and anti-neutrophil cytoplasmic antibodies–associated vasculitis. MN is one of the most common associated lesions of anti-GBM disease. The combination of the two diseases is well documented since the first report in 1974,[8] with either MN followed by anti-GBM disease or simultaneous findings of the two diseases.[9–15]Occasionally, the converse chronological order was reported in individual cases.[16–19] However, the clinical features and outcomes of these patients are still not clear.

The immunological characteristics of autoantibodies have been proven to be of significant clinical importance. Anti-GBM antibodies with broader antigen spectrum are closely associated with higher levels of serum creatinine and more crescents in glomeruli.[20] Anti-α3(IV)NC1 immunoglobulin G1 (IgG1) and IgG3 are more frequently detected in patients with severe renal dysfunction.[21] In primary MN, recent studies have indicated that the binding of autoantibodies against target antigens on podocytes such as M-type phospholipase A_2 receptor (PLA_2R) initiates the disease process. However, the immunological characteristics of autoantibodies and their target antigens in patients with

both lesions have not been explored. The current study was designed to investigate the clinical and immunological features of anti-GBM patients with MN and to provide insight into the pathogenesis of this rare entity.

RESULTS

Clinical and histological data of patients with combined anti-GBM disease and MN

Eight patients with combined anti-GBM disease and MN were enrolled in this study. The demographic and clinical data are shown in Table 1. Seven patients were male and one was female, with a mean age of 31.6 ± 9.7 years. Among them, five (62.5%) patients had hemoptysis and six (75%) presented with crescentic glomerulonephritis.

Compared with the classical anti-GBM patients without MN, these eight patients presented significantly lower level of serum creatinine on diagnosis (523.6 ± 351.9 vs. 916.3 ± 345.2 μmol/l, $P = 0.007$), lower proportion of oliguria/anuria (0 vs. 50%, $P = 0.013$) and gross hematuria (0 vs. 50%, $P = 0.013$), and a higher level of urinary protein excretion (7.9 ± 5.0 vs. 3.6 ± 2.8 g/24 h, $P = 0.045$) (Table 1).

Renal biopsies were performed on all patients and the pathological data were reviewed independently by two renal pathologists. Immunofluorescence staining performed on renal biopsies from the eight patients showed granular capillary loop IgG staining with intensity $>1+$ ($-$ to $4+$). The linear staining of GBM was occasionally discerned. IgA and IgM were negative. In addition, biopsies from these patients showed fewer crescents in glomeruli (65.5 ± 32.4 vs. 88.1 ± 18.1%), though the difference did not reach statistical significance (Table 1). The extent of glomerular sclerosis, mesengial proliferation, and tubular-interstitial damage was assessed and scored semi-quantitatively, as shown in Table 2. Six of the eight patients were found in stage I of MN and two in stage II. All of the eight patients had interstitial infiltration of leukocytes and five of them (62.5%) scored $\geqslant 2$, which is not characteristic of typical MN. Despite these data, no significant correlation was found between the histological parameters and the clinical data in these patients.

Of the eight anti-GBM patients with MN, five patients (62.5%) recovered their renal function after treatment and three remained dialysis dependent (Table 1). In patients with classical anti-GBM disease, 26/30 (86.7%) patients progressed to end-stage kidney disease. Using log-rank tests and Kaplan–Meier curves for univariate survival analysis, we determined that patients with MN had better renal outcome than those without MN ($P = 0.033$; Figure 1). No significant difference was observed between patient survivals of the two groups ($P > 0.05$).

Target antigens of circulating anti-GBM antibodies

The target antigens of anti-GBM antibodies were examined using recombinant human α1 to α5(IV)NC1. Sera from all eight patients with combined anti-GBM disease and MN recognized α3(IV)NC1, while sera reactivity to other α chains was varied. Sera of two patients (25%) reacted with α1(IV)NC1, one (12.5%) reacted with α2(IV)NC1, and three (37.5%) with α5(IV)NC1. Sera from all eight patients did not react with α4(IV)NC1. In comparison, sera from patients with classical anti-GBM disease possessed antibodies against all the five α chains with 60.0% of the sera (18/30) reactive to α1(IV)NC1, 53.3% (16/30) to α2(IV)NC1, 100% to α3(IV)NC1, 13.3% (4/30) to α4(IV)NC1, and 76.7% (23/30) to α5(IV)NC1, respectively. No serological reactivity against the five α chains was detected in the 20 patients with primary MN.

The target antigen spectrum was narrower in patients with combined anti-GBM disease and MN. In all, 5/8 (62.5%) sera recognized α3(IV)NC1 alone, while most sera (24/30, 80%) from the patients with classical anti-GBM disease recognized more than one type of α chains ($P = 0.031$).

Table 1 | Clinical and pathological features and renal outcomes of anti-GBM patients with and without membranous nephropathy

	With MN ($n = 8$)	Without MN ($n = 30$)	P
Age (years)	31.6 ± 9.7	32.8 ± 15.4	0.839
Gender (male/female)	7/1	26/4	1.000
Prodromal infection, % (*n*)	62.5 (5/8)	50 (15/30)	0.697
Hydrocarbon exposure, % (*n*)	0 (0/8)	23.3 (7/30)	0.308
Smoking, % (*n*)	75 (6/8)	50 (15/30)	0.423
Hemoptysis, % (n)	62.5 (5/8)	50.0 (15/30)	0.697
Hemoglobin (g/l)	100.4 ± 36.5	81.1 ± 27.9	0.127
Oliguria/anuria, % (*n*)	0 (0/8)	50.0 (15/30)	**0.013**
Urinary protein (g/24 h)	7.9 ± 5.0	3.6 ± 2.8	**0.045**
Gross hematuria, % (*n*)	0 (0/8)	50 (15/30)	**0.013**
Serum creatinine on diagnosis (μmol/l)	523.6 ± 351.9	916.3 ± 345.2	**0.007**
Levels of anti-GBM antibodies (U/ml)	72.3 ± 50.1	78.4 ± 38.6	0.483
Percentage of crescents in glomeruli (%)	65.5 ± 32.4	88.1 ± 18.1	0.052
Treatment, % (n)			
Plasma exchange	62.5 (5/8)	60.0 (18/30)	1.000
Prednisone	87.5 (7/8)	90.0 (27/30)	1.000
Cyclophosphamide	75.0 (6/8)	73.3 (22/30)	1.000
Renal survival at 1 year, % (*n*)	62.5 (5/8)	13.3 (4/30)	0.010

Abbreviations: GBM, glomerular basement membrane; MN, membranous nephropathy.
Bold values represent $P < 0.05$.

Epitope specificity of circulating antibodies against α3(IV)NC1

The epitope specificity of anti-α3(IV)NC1 antibodies were examined using three chimeric proteins, containing epitopes of E_A, E_B, and non-E_{AB}. All sera (100%) from patients with combined anti-GBM disease and MN reacted with E_A and seven (87.5%) reacted with E_B. No significant difference of E_A and E_B recognition was observed between the two groups of patients ($P > 0.05$; Table 3).

Although the levels of anti-E_A antibodies were comparable between the two groups, the levels of antibodies against E_B were significantly lower in patients with combined anti-GBM disease and MN than that in patients without MN (optical density (OD) value: 0.46 ± 0.54 vs. 0.77 ± 0.47, $P = 0.032$; Table 3).