Glomerulonephritis with coexistent immune deposits and antibasement membrane activity

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SUMMARY Six patients with coexistent antiglomerular basement membrane disease and granular immunoreactants in the glomerular basement membrane and mesangium are discussed. These six patients represent 35% of all patients with antiglomerular basement membrane nephritis examined over 10 years. All patients presented with acute, oliguric renal failure and rapid deterioration in renal function. In all patients the pathogenetic role of the antiglomerular basement membrane antibody was confirmed by the demonstration of linear deposits of IgG along the glomerular basement membrane and antiglomerular basement membrane antibody activity in the serum or renal eluates, or both. Evidence for the existence of concurrent immune aggregates was obtained by immunofluorescence studies and electron microscopy. Radioimmunoassays, which were performed in two patients to detect circulating immune complexes, however, yielded negative results. The possible mechanisms concerned in the evolution of this condition and their potential implications are reviewed.

Two major nephritogenic immune mechanisms have been implicated in the causation of human glomerulonephritis: the specific interaction of antibodies with antigens that are intrinsic to the glomerular basement membrane (GBM) and the lodging of circulating, nonglomerular, antigen-antibody complexes in the glomerulus. Traditionally, glomerulonephritis has been thought to result from either one of these mechanisms, but not both. Anti-GBM nephritis comprises about 2-5%, and immune complex nephritis about 70-80% of all immunologically mediated glomerulonephritis.^{1,2} Although much information has been gathered about the pathogenesis of anti-GBM disease, little information is available on the factors that are responsible for the induction of anti-GBM antibody. The inducing factors are probably heterogeneous and may include injury to the capillary wall, as in some cases of idiopathic membranous glomerulonephritis^{3,4}; penicillamine treatment⁵; viral influenza⁶; other associated GBM disease (for example Nail-Patella syndrome)⁷; volatile hydrocarbon exposure⁸; traumatic renal cortical necrosis⁹;

and Hodgkin's and non-Hodgkin's lymphomata.¹⁰

Recently, a few patients with features of both immune complex nephritis and anti-GBM antibody mediated nephritis have been described,¹¹⁻¹⁷ raising the possibility of yet another range of clinicopathological entities. This report describes the immunopathological features of six patients who had both anti-GBM disease and granular immunoreactants in glomeruli.

Material and methods

A total of 1956 consecutive renal biopsy specimens were examined over 10 years from 1972 to 1982. Seventeen specimens (0.9%) fulfilled the clinical, morphological, and immunopathological criteria for anti-GBM disease.¹⁴ Six of these 17 (35%) also showed evidence of deposition of immunoreactants in the kidneys. These six cases are discussed in this report. Whole kidneys were also available for study in four of these patients.

The procedures for processing tissue for light, immunofluorescence, and electron microscopy; the controls for ascertaining the specificity of the reagents used; and the instruments for assessing tissue changes were similar to those described in an earlier

report.¹⁸ An average of 47 glomeruli (range eight to 100) were studied by light and immunofluorescence microscopy. For immunofluorescence studies the sections were stained with antisera to human IgG, IgM, IgA, C1q, C3, C4, fibrin-related-antigens, albumin, and alpha-2-macroglobulin. An arbitrary scale of 0–3+ was used to indicate the intensity of fluorescent deposits.¹⁹ One to three glomeruli from each biopsy were studied ultrastructurally.

Anti-GBM activity in the sera and renal eluates was assessed by an indirect immunofluorescence assay.¹⁹ Renal eluates were obtained by extraction in citrate buffer (pH 3.2).¹⁹ Radioimmunoassays to detect anti-GBM antibody in the sera and renal eluates from four patients, and radioimmunoassays (C1q binding assay and Raji cell assays) to detect circulating immune complexes in the sera of two patients, were performed by Dr Curtis Wilson.

Some observations on three of our patients (cases 1, 2, and 3) have already been published.¹⁹

Results

The six patients (five men and one woman) were between 21 and 70 years of age with a mean age of 54 years (Table 1). All patients had had manifest renal disease for only a short period (1–12 weeks). All patients presented with acute oliguric renal failure and rapid deterioration in renal function that required dialysis. In addition, two patients presented with dyspnoea and haemoptysis. All patients exhibited a nephritic urinary sediment, with slight proteinuria, and five were hypertensive (diastolic pressure > 90 mm Hg). None of these patients had the nephrotic syndrome. Serum C3 or C4, or both, was measured in three patients, and two had normal complement concentrations. A low serum C3 con-

Fig. 1 Light microscopic appearance of a typical glomerulus showing cellular crescent compressing the capillary tuft. The interstitium exhibits a dense inflammatory infiltrate. Periodic acid methanamine silver stain. $\times 280$.

centration, along with a high antistreptolysin O titre, was found in one patient (case 2).

Three patients died as a result of extrarenal complications (cases 1, 3, and 5), one of them after renal transplantation (case 3). No patient had vasculitis. Other features are summarised in Table 1.

LIGHT MICROSCOPY

All six biopsy specimens showed classic histopathological features of crescentic glomerulonephritis (Fig. 1 and Table 2).

IMMUNOPATHOLOGY

The immunofluorescence findings are summarised in Table 3. Biopsy specimens from all six patients

Table 1 Summary of clinical and laboratory findings

Case no	Age/Sex	Disease duration (wk)	Blood pressure (mm Hg)	Creatinine/blood urea nitrogen (mg/100 ml)	C3/C4 (mg/100 ml)	Anti-GBM serum/eluate	Antistreptolysin O titre	Additional information
1	63/M	1–2	155/90	18/132	ND	+/+	<100 U Todd	Acute myocardial infarction 6 weeks earlier. Rupture of aortic aneurysm. Died.
2	21/M	1	160/95	7/67	30/ND	+/+	1200 U Todd	Rheumatic fever 10 years and 3 years earlier. Sore throat 1 month before admission. Nephrectomy.
3	40/M	4	180/90	10/117	116/ND	+/+	333 U Todd	Nephrectomy. Renal transplantation. Died from perforated peptic ulcer 3 months later.
4	70/M	12	155/90	14/115	98/38	+/-ND	ND	Dyspnoea. Haemoptysis.
5	64/M	1–2	150/90	15/250	ND/ND	+/+	<100 U Todd	Haemoptysis. Perforation of sigmoid colon. Peritonitis.
6	63/F	1	180/80	16/30	ND/ND	+/-ND	ND	Septic shock. Died. Perforated bowel.

ND = Not done.

Table 2 *Light microscopic findings*

Features	No positive	% Average (range)
Glomerular epithelial and fibroepithelial crescents	6/6	68% (16%–100%)
Glomerular obsolescence	6/6	25% (4%–76%)
Neutrophils in glomeruli	6/6	
Multinucleated cells in glomeruli	1/6	
Fibrin (glomeruli and crescents)	6/6	
Tubular atrophy, interstitial fibrosis, and chronic inflammation	6/6	
Thickening of vascular walls	2/6	

showed sharp, continuous, linear deposits of IgG (3+) (Fig. 2), and all but one had IgM deposits (1+) along the GBM. The accompanying C3 deposits were similarly linear (1–3+) in three biopsy specimens, whereas interrupted, short linear deposits (1–2+) were seen in one biopsy specimen, and short linear and granular deposits (2+) were noted in another; exclusively granular deposits of C3 (1+) were seen in a single biopsy specimen (Fig. 3). All the C3 deposits were found mostly along the capillary basement membranes. Linear and short linear deposits of C1q (1–2+) were seen in two biopsy specimens. Linear deposits of C4 were observed in one and both short linear and granular deposits were found in another (1–2+). Fibrin related antigens (2–3+) were observed within the crescents in all the specimens. No IgA, albumin, or alpha-2-macroglobulin deposits were found in the glomeruli.

Information on anti-GBM activity in sera and renal eluates is given in Table 1. Circulating anti-GBM antibody was detectable in the sera of five patients by indirect immunofluorescence assay and in the sera of all three patients tested by radioimmunoassay. Anti-GBM activity was found in all of the four eluates that were tested. Radioimmunoassay for circulating immune complexes performed on sera from two patients (cases 5 and 6) yielded negative results.

ELECTRON MICROSCOPY

The ultrastructural findings are summarised in Table 4. Scattered, definitive, electron dense deposits conforming to immunoreactants were seen in a random

Fig. 2 *Immunofluorescent photomicrograph showing a glomerulus exhibiting linear IgG staining along the glomerular capillary basement membranes. × 320.*

Fig. 3 *Immunofluorescent photomicrograph showing predominantly granular deposits of C3 along the glomerular capillary basement membranes. × 320.*

Table 3 *Direct immunofluorescence microscopy findings in glomeruli*

Case no	IgG	IgM	C3	C1q	C4
1	3+(L)	1+(L)	3+(L)	Negative	
2	3+(L)	1+(L)	3+(L)	Negative	
3	3+(L)	1+(L)	1+(L)	1+(L)	
4	3+(L)	Negative	2+(SL and G)		2+(SL and G)
5	3+(L)	1+(L)	1+–2+(SL)	1+–2+(SL)	1+(L)
6	3+(L)	1+(SL)	1+(G)	Negative	Negative

IgA, albumin and alpha-2-macroglobulin were not present in any of the specimens. Fibrin (2–3+) was observed within the crescents in all the biopsies. L = linear, SL = short linear, G = granular.

Fig. 4 Electron photomicrograph illustrating subendothelial electron dense deposits (D) along duplicated basement membrane. $\times 15\,400$.

Fig. 5 Electron photomicrograph illustrating a large subepithelial deposit. $\times 10\,850$.

distribution (Fig. 4 and 5). Subepithelial deposits were found in three of the six specimens, subendothelial deposits in three of them, intramembranous deposits in five, and mesangial deposits in three. The patterns of deposition did not correspond to any typical category of immune complex mediated nephritis. The uninvolved basement membranes showed non-specific features commonly seen in uncomplicated anti-GBM disease.

Discussion

Anti-GBM disease comprises about 2–5% of immunologically mediated human glomerulonephritides. Diagnosis rests on detection of antibodies bound to the GBM in a continuous, linear configuration and demonstration of anti-GBM activity in sera or renal eluates, or both.

In the past decade several reports have emphas-

Table 4 Findings on electron microscopy

Case no	Electron dense deposits	Other ultrastructural features (common to all biopsies)
1	Subepithelial Subendothelial Intramembranous Mesangial	Glomerular basement membrane Thickening Attenuation Wrinkling Disruption Rarefaction Loss of demarcation of lamina rara and densa
2	Intramembranous	
3	Subendothelial	Formation of basement membrane like material
4	Intramembranous	Capillary loop collapse
5	Subendothelial	
5	Intramembranous	Cellular crescents
6	Mesangial	
6	Subepithelial	
6	Subepithelial	Fibrin in the crescents
6	Mesangial	

ised the pronounced heterogeneity in the clinical manifestations and course of anti-GBM disease.²⁰⁻²² The clinical manifestations, for example, range from minimal renal dysfunction to a rapidly progressive clinical course and also include a few instances of spontaneous remission or, rarely, persistence of the pathogenetic mechanism for more than a year. The heterogeneity of the inducing or associated factors has also been emphasised. Moorthy *et al*,¹⁴ for example, reported three patients with anti-GBM nephritis superimposed on a pre-existing membranous nephropathy; they postulated that the intermingling of immune complexes with newly formed basement membrane material might have altered the antigenicity of the basement membrane, leading to the production of anti-GBM antibody. The reverse sequence—namely, the superimposition of an immune complex disease on anti-GBM nephritis or the induction of an immune complex glomerulonephritis with basement membrane antigens—has also been postulated.¹³ This proposed sequence is in contrast to that reported by Moorthy *et al*¹⁴ and Klassen *et al*,⁴ (in which anti-GBM nephritis occurred in a pre-existing membranous nephropathy) in that anti-GBM nephritis and membranous nephropathy are hypothesised to have been produced simultaneously by a mechanism analogous to one initiated by heterologous nephrotoxic antibodies²³ or by autologous anti-GBM antibodies (such as those that have been produced in experimental animals).²⁴ Serological evidence of circulating immune complexes superimposed on pre-existing anti-GBM disease has been put forward by Pussell *et al*¹² and Vanhille *et al*,¹⁵ where the circulating immune complexes appearing late in the course of the disease allegedly contributed to worsening of organ disease (lung and kidney) and the patient's clinical condition.

The six patients reported here had documented anti-GBM disease as shown by (a) rapidly progressive (crescentic) glomerulonephritis, (b) linear IgG deposits along the GBM by immunofluorescence, and (c) anti-GBM antibodies in the sera or eluates, or both. In addition, immunofluorescence and ultrastructural studies indicated the presence of associated granular deposits of immunoreactants along the GBM or the mesangium, or both, in all six biopsy specimens, although supportive serological evidence of circulating immune complexes was not obtained in the two patients (cases 5 and 6) whose sera were tested by the C1q binding assay and the Raji cell assay. Immunofluorescence showed granular deposits in only two of these patients.

The temporal relation between the deposition of immunoreactants and anti-GBM antibody production in our patients could not be ascertained because

serial serological assays for anti-GBM antibody and circulating immune complexes were not performed. Only one patient (case 2), however, had a clinical course that was complicated by an infectious episode. The streptococcal infection in this patient could represent a coincidence, but speculation as to the role of streptococcal antigens binding to and altering the antigenicity of the GBM seems theoretically possible. None of the other five patients had a clinical history or laboratory findings that suggested an antecedent viral or bacterial infection, and none had a pre-existing glomerular disease. Thus it is not likely that immune complexes associated with infection provided the necessary conditions for a grafted immune complex disease in those five patients. On the other hand it is possible that fixation of anti-GBM antibody in the GBM might augment or facilitate the glomerular deposition of circulating immune complexes. Such a synergistic effect has been shown in animal models by Trevillian and Cameron.²⁵ It is also conceivable that anti-GBM antibodies might bind to intact GBM antigen(s) as well as to freed, entrapped, GBM antigens, the latter to form an immune aggregate and result in the granular immunofluorescent staining pattern which we observed in our biopsy specimens. Such a hypothesis is supported by the findings of Jennette *et al*,¹⁶ who reported findings of concurrent anti-GBM disease and immune complex in a biopsy specimen in which the linear and granular deposits showed an IgG subclass restriction (IgG1 and IgG4) similar to that found in 13 other cases of uncomplicated anti-GBM antibody disease. It is also possible that the immune aggregates are composed of rheumatoid factors or anti-idiotypic antibodies and that the binding takes place in situ (in the damaged glomerular capillary wall or mesangia). Presumptive evidence for such a mechanism has been found by Sugisaki *et al*²⁶ in their investigation of various forms of glomerulonephritis mediated by immune complexes. The two latter possibilities would also explain the failure to detect circulating immune complexes in the sera of these patients.

Our study focused on an interesting and rare finding that may bear important pathogenetic connotations that are, as yet, undefined. We suspect that with increasing awareness similar pathological entities will be identified more often, and we propose that prospective investigative studies should include serial assays of anti-GBM antibody and circulating immune complexes in the serum to define the temporal relation of this association. If we assume that circulating immune complexes can be detected and isolated, then the use of techniques such as polyacrylamide gel electrophoresis and transfer of immunoreactants to nitrocellulose mem-

branes might afford an opportunity to gain some insight into the antigen(s) and antibodies that are concerned. Until then, and also until the specificity of the antibody to the putative antigen(s) has been determined, the association of anti-GBM disease and immune complex nephritis will remain a clinical curiosity to challenge the immunopathologist.

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