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NEPHROLOGY FORUM

Rapidly progressive crescentic glomerulonephritis

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CASE PRESENTATION

A 77-year-old white woman was found unresponsive on the floor of her home. Emergency medical technicians were called, and she was transported to the University of North Carolina Hospitals. She recovered consciousness and gave a history of recent anorexia, lower extremity weakness and paresthesias, and difficulty walking. Her medical history was significant for rectal adenocarcinoma 9 years earlier that was successfully treated with radiation, chemotherapy, and partial colectomy. A recent colonoscopy showed no recurrence. She had been diagnosed with a myeloproliferative disorder with features of essential thrombocytosis, and hydroxyurea (500 mg/day) had been initiated.

Physical examination revealed an elderly, thin, lethargic woman who was afebrile and had a blood pressure of 140/60 mm Hg, guaiac-negative stool, no rash, 1+ pitting edema to the knees, and bilateral foot drop. Computerized tomography (CT) identified a small subdural hematoma. Laboratory data included blood urea nitrogen (BUN), 158 mg/dL; serum creatinine, 10.8 mg/dL (0.6 mg/dL 2 months prior to admission); potassium, 6.3 mmol/L; glucose, 101 mg/dL; albumin, 2.3 g/dL; white blood cell count, 23.3×10^9/L (neutrophil count, 19.3×10^9/L); red blood cell count, 3.5×10^{12}/L; and platelet count, 799×10^9/L. Urinalysis revealed 2+ protein, 4+ blood, and numerous dysmorphic red blood cells. Chest radiographs showed pulmonary edema. Hemodialysis was initiated and continued throughout her admission. Renal ultrasound showed normal kidney size with echogenicity consistent with parenchymal disease. Serologic tests were ordered and a renal biopsy was performed.

The renal biopsy demonstrated involvement of essentially all glomeruli by necrosis, sclerosis, or both. Approximately 90% of glomeruli had cellular or fibrocellular crescents (Fig. 1A). There was focal disruption of Bowman's capsule. Most glomeruli had prominent adjacent periglomerular inflammation, including occasional multinucleated giant cells. A few small foci of granulomatous inflammation were present but were centered on identifiable glomeruli (Fig. 1C). Arteries and arterioles had moderate sclerosis as well as focal necrotizing vasculitis (Fig. 1D). Immunofluorescence microscopy demonstrated intense linear staining of glomerular basement membranes (GBMs) for immunoglobulins (IgG) (Fig. 1B), kappa light chains, and lambda light chains, and moderate granular staining for C3. There was no staining for IgA, IgM, or C1q. There was marked staining of crescents for fibrin. Electron microscopy revealed extensive disruption of glomerular basement membranes and Bowman's capsule, segmental fibrinoid necrosis, and no immune-complex-type electron-dense deposits.

Serology demonstrated the presence of high-titer myeloperoxidase-specific antineutrophil cytoplasmic autoantibodies (MPO-ANCA) and high-titer anti-GBM antibodies. Testing for proteinase 3–specific ANCA (PR3-ANCA) and antinuclear autoantibodies (ANA) was negative.

The patient received hemodialysis, plasmapheresis, and pulse methylprednisolone. Cyclophosphamide was not instituted because of the poor prognosis for recovery of renal function and the overall frail status of the patient. During the entire 3-week admission, she produced less than 50 mL urine/day. Her lower extremity neuropathy improved and her lungs cleared. After Permacath placement, she was discharged to a nursing home on low-dose oral prednisone and scheduled to receive hemodialysis.

DISCUSSION

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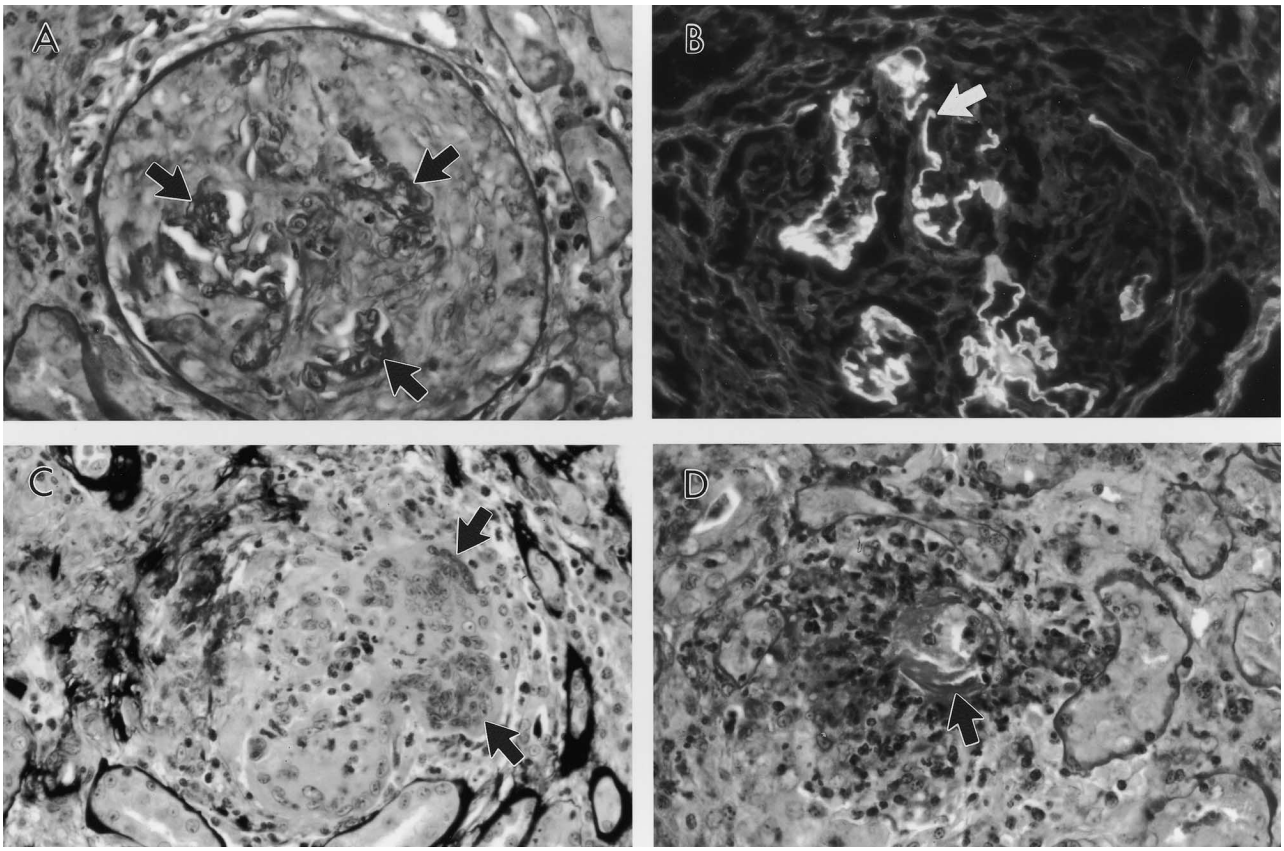

Fig. 1. Pathologic findings in the renal biopsy specimen. (*A*) Glomerular cellular crescents with fragmentation of the glomerular tuft (arrows) (periodic acid-Schiff stain, ×600). (*B*) Linear staining of glomerular basement membranes for IgG showing numerous breaks in the basement membrane (arrow) (FITC anti-IgG stain, ×600). (*C*) Focal periglomerular, interstitial granulomatous inflammation with multinucleated giant cells (arrows) (Jones silver methenamine stain, ×600). (*D*) Necrotizing arteritis with fibrinoid necrosis (arrow) and surrounding infiltration by predominantly neutrophilic leukocytes (periodic acid-Schiff stain, ×600).

disease and glomerular crescents in 1914. They called the clinical expression "subacute nephritis" and the renal lesion "extracapillary nephritis." In 1942, Ellis [2] referred to this aggressive category of glomerulonephritis as "rapidly progressive type I nephritis" and provided a photomicrograph of a glomerulus with "epithelial crescent formation" to illustrate the underlying pathologic lesion.

Crescentic glomerulonephritis must be diagnosed promptly and precisely so that appropriate treatment can be initiated as quickly as possible. The best predictor of outcome for all types of crescentic glomerulonephritis is the severity of renal failure at the time therapy begins. Even several days' delay in diagnosis and treatment can have a major negative impact on outcome because of the rapidly progressing loss of renal function that typically accompanies crescentic glomerulonephritis. Requirements for satisfactory management include a proper index of suspicion by the physician who first encounters the patient, prompt referral to a nephrologist, timely and accurate clinical and pathologic diagnosis, rapid institution of appropriate immunosuppressive therapy, and proper long-term management of persistent or recurrent disease and its chronic sequelae. Current therapy is relatively crude and employs nonspecific and toxic anti-inflammatory and immunosuppressive agents. A better understanding of the pathophysiology of crescentic glomerulonephritis should lead to more effective, more specific, and less toxic therapies.

The patient described exemplifies an unfortunate presentation and outcome because the crescentic glomerulonephritis was far advanced when first recognized and thus not responsive to treatment. The glomerulonephritis itself illustrates an uncommon but well-recognized concurrence of circulating ANCA and anti-GBM antibodies in a patient with crescentic glomerulonephritis.

Immunopathologic categories

The pathology and immunopathology of crescentic glomerulonephritis comprise three major categories: (*1*) anti-GBM crescentic glomerulonephritis, (*2*) immune-complex crescentic glomerulonephritis, and (*3*) pauci-immune crescentic glomerulonephritis [3, 4]. Unlike immune-complex glomerulonephritis and anti-GBM glomerulonephritis, pauci-immune crescentic glomerulonephritis

Table 1. Frequency of different types of crescentic glomerulonephritis in consecutive native renal biopsy specimens evaluated by the University of North Carolina Nephropathology Laboratory

	Pauci-immune crescentic glomerulonephritis[a]	Immune-complex crescentic glomerulonephritis[b]	Anti-GBM crescentic glomerulonephritis[c]	Other crescentic glomerular disease[d]
All ($N = 632$)	60% 377/632	24% 154/632	15% 92/632	1% 9/632
Age 1 to 20 years ($N = 73$)	42% 31/73	45% 33/73	12% 9/73	0% 0
Age 21 to 60 years ($N = 303$)	48% 145/303	35% 106/303	15% 44/303	3% 8/303
Age 61 to 100 years ($N = 256$)	79% 201/256	6% 15/256	15% 39/256	0% 1/256

[a] Pauci-immune crescentic glomerulonephritis was defined as glomerulonephritis with 50% or more crescents and 2+ or less staining of glomeruli for any immunoglobulin

[b] Immune-complex crescentic glomerulonephritis was defined as glomerulonephritis with 50% or more crescents and greater than 2+ nonlinear glomerular staining for any immunoglobulin by direct immunofluorescence microscopy (plus membranoproliferative and postinfectious glomerulonephritis)

[c] Anti-GBM crescentic glomerulonephritis was defined as glomerulonephritis with 50% or more crescents and 2+ or greater linear GBM staining for immunoglobulins by direct immunofluorescence microscopy

[d] The "other" category includes all other glomerular diseases with 50% or more crescents, such as thrombotic microangiopathy, diabetic glomerulosclerosis, and monoclonal immunoglobulin deposition disease

by definition has an absence or paucity of glomerular staining for immunoglobulins [4, 5]. In approximately 80% of patients, pauci-immune crescentic glomerulonephritis is associated with ANCA and thus can be called ANCA-associated crescentic glomerulonephritis [4, 6]. A few patients with crescentic glomerulonephritis have concurrent expression of more than one immunopathologic phenotype. Approximately one-half of patients with anti-GBM glomerulonephritis have pulmonary capillaritis (Goodpasture's syndrome), and approximately three-fourths of patients with ANCA-glomerulonephritis have systemic small-vessel vasculitis (for example, microscopic polyangiitis or Wegener's granulomatosis). Immune-complex crescentic glomerulonephritis has the lowest frequency of systemic vasculitis, although it can occur (for example, with Henoch-Schönlein purpura). Also, some patients have overlap between immunopathologic phenotypes. For example, approximately one-fourth to one-third of patients with anti-GBM glomerulonephritis have ANCA as well, like the patient presented today.

In a patient with rapidly progressive glomerulonephritis, statistically the most likely diagnosis is ANCA-associated pauci-immune crescentic glomerulonephritis unless the patient is a child (Table 1). In children, immune-complex crescentic glomerulonephritis is most common because of the combined effect of less-frequent ANCA disease and a higher frequency of most types of immune-complex glomerulonephritis, including acute post-streptococcal glomerulonephritis, Henoch-Schönlein purpura nephritis, IgA nephropathy, membranoproliferative glomerulonephritis, and lupus nephritis. ANCA glomerulonephritis is by far the most common cause of rapidly progressive glomerulonephritis in adults, especially older adults. Approximately 80% of crescentic glomerulonephritis in patients over 60 years of age is pauci-immune disease, which is associated with ANCA approximately 80% of the time. Anti-GBM disease is uncommon at any age.

Approximately three-fourths of patients with pauci-immune or ANCA-associated crescentic glomerulonephritis have systemic small-vessel vasculitis [6–9]. The pauci-immune glomerulonephritis in patients with no evidence for systemic vasculitis sometimes is called "renal-limited vasculitis" because it is pathologically identical to the glomerulonephritis in patients with concurrent vasculitis elsewhere. The three clinicopathologic categories of ANCA-associated, systemic, small-vessel vasculitis are microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome [7]. A diagnosis of microscopic polyangiitis is appropriate in the setting of small-vessel vasculitis with no evidence for granulomatous inflammation or asthma. A diagnosis of Wegener's granulomatosis is appropriate if granulomatous inflammation is present (usually in the respiratory tract) but not asthma. Finally, a diagnosis of Churg-Strauss syndrome is appropriate with granulomatous inflammation, eosinophilia, and asthma. For the diagnostic categorization of systemic vasculitis, periglomerular granulomatous inflammation does not fulfill the granulomatous inflammation criterion.

In patients with ANCA-associated glomerulonephritis, most ANCA have specificity for MPO or PR3, which are proteins in the primary granules of neutrophils and the lysosomes of monocytes [6]. Patients with Wegener's granulomatosis have the highest frequency of PR3-ANCA, and patients with renal-limited disease have the highest frequency of MPO-ANCA [6–9].

Anti-GBM glomerulonephritis is the most aggressive form of glomerulonephritis, with the highest frequency of renal insufficiency and the highest frequency of crescent formation at the time of diagnosis (Tables 2 and 3). More than 95% of patients with anti-GBM glomerulonephritis have crescents at the time of biopsy, and approximately 85% have 50% or more of glomeruli with crescents (Table 3). ANCA glomerulonephritis is a close second; approximately 90% of patients have crescents, and approximately 50% have 50% or more of glomeruli with crescents. In contrast, all types of immune-complex

Table 2. Features of different types of crescentic glomerulonephritis evaluated by the University of North Carolina Nephropathology Laboratory[a]

	Mean age *range*	Male:female	Black:White[b]	Creatinine *mg/dL* (range)	Proteinuria *g/24 hours* (range)
Anti-glomerular basement membrane (GBM) crescentic glomerulonephritis	52 ± 21 (14 to 84) *N* = 92	1.0:1.0 45:47 *N* = 92	1.0:9.0 7:63 *N* = 70	9.7 ± 7.2 (0.8 to 50) *N* = 86	1.67 ± 3.35 (0.20 to 16.20) *N* = 68
Pauci-immune crescentic glomerulonephritis	56 ± 20 (2 to 92) *N* = 377	1.0:0.9 202:177 *N* = 379	1.0:3.7 65:239 *N* = 304	6.5 ± 4.0 (0.8 to 22.1) *N* = 338	1.94 ± 2.95 (0.11 to 18.00) *N* = 331
Immune-complex crescentic glomerulonephritis	33 ± 17 (4 to 77) *N* = 154	1.0:1.6 61:95 *N* = 156	1.0:0.9 67:62 *N* = 129	4.9 ± 3.8 (0.8 to 21.7) *N* = 145	4.39 ± 4.77 (0.30 to 22.00) *N* = 136

[a] Anti-GBM crescentic glomerulonephritis, pauci-immune crescentic glomerulonephritis, and immune-complex crescentic glomerulonephritis were defined as detailed in Table 1

[b] Approximate black:white ratio in the referral population is 1:3

Table 3. Frequency of glomerular crescents, necrosis, and endocapillary hypercellularity in different types of glomerular disease evaluated by the University of North Carolina Nephropathology Laboratory

Type of glomerular disease	Number	% with any crescents	% with >50% crescents	Average % glomerular crescents[b]	Glomerular necrosis (0 to 4+)	Glomerular hypercellularity[c] (0 to 4+)
Anti-GBM glomerulonephritis	105	97.1	84.8	77	1.7+	0.8+
ANCA glomerulonephritis[a]	181	89.5	50.3	49	1.2+	0.8+
Lupus glomerulonephritis (III & IV)	784	56.5	12.9	31	1.7+	2.2+
H-S purpura glomerulonephritis	31	61.3	9.7	27	0.4+	1.5+
IgA nephropathy	853	32.5	4.0	21	0.1+	1.4+
Post-infectious glomerulonephritis	120	33.3	3.3	19	0.3+	2.7+
Type I membranoproliferative glomerulonephritis	307	23.8	4.6	25	0.2+	2.8+
Type II membranoproliferative glomerulonephritis	16	43.8	18.8	48	0.2+	1.8+
Fibrillary glomerulonephritis	101	22.8	5.0	26	0+	0.6+
Monoclonal immunoglobulin deposition disease	54	5.6	0	13	0	0.3
Thrombotic microangiopathy	251	5.6	0.9	26	0.4+	0.3+
Diabetic glomerulosclerosis	648	3.2	0.3	20	0+	0.3+
Nonlupus membranous glomerulopathy	1092	3.2	0.1	15	0.0	0.1

[a] ANCA glomerulonephritis was defined as glomerulonephritis with 2+ or less staining of glomeruli for any immunoglobulin in a patient who is positive for MPO-ANCA or PR3-ANCA by ELISA

[b] Average % of glomeruli with crescents when crescents were present

[c] Endocapillary (not extracapillary) hypercellularity

glomerulonephritis have a much lower frequency of crescent formation and, when crescents are present, they rarely affect 50% or more of glomeruli.

Patients with anti-GBM disease can have anti-GBM glomerulonephritis alone or in combination with anti-GBM, antibody-mediated pulmonary hemorrhage (Goodpasture's syndrome). Approximately 40% to 60% of patients with anti-GBM disease have pulmonary hemorrhage [10, 11]. Other factors such as pulmonary alveolar capillary damage by cigarette smoke or other hydrocarbon exposure might predispose patients to lung involvement [12, 13]. Cigarette smoke likely predisposes to lung involvement via oxidant-induced neutralization of alveolar alpha 1-antiproteinase and resultant greater susceptibility to capillary injury by unopposed proteinases released by activated leukocytes [14].

Glomerulonephritis accompanied by pulmonary hemorrhage (pulmonary-renal syndrome) raises the possibility of ANCA-associated small-vessel vasculitis as well as anti-GBM antibody-mediated Goodpasture's syndrome. The most common cause of pulmonary-renal syndrome is ANCA-associated small-vessel vasculitis, such as microscopic polyangiitis or Wegener's granulomatosis. In a study of patients with pulmonary-renal syndrome, Niles et al [15] found that 54% had ANCA, 7% had ANCA and anti-GBM antibodies, and 6% had anti-GBM alone.

The best laboratory predictors of pauci-immune crescentic glomerulonephritis and anti-GBM crescentic glomerulonephritis are serologic detection of ANCA or anti-GBM antibodies, respectively [6–9, 16–18]. The best laboratory predictors of immune-complex crescentic glomerulonephritis are various serologic markers for different types of immune-complex disease, for example, hypocomplementemia, antinuclear antibodies, cryoglobulins, or antibodies indicative of a potentially nephritogenic infection. At the University of North Carolina, 78% of

Table 4. Comparison of pathologic and clinical features of glomerulonephritis in patients (with complete serologic data and glomerulonephritis with or without crescents) with different ANCA and anti-glomerular basement membrane (GBM) serology findings evaluated by the University of North Carolina Nephropathology Laboratory

	MPO-ANCA positive, anti-GBM negative $N = 102$	PR3-ANCA positive, anti-GBM negative $N = 52$	ANCA positive, anti-GBM positive $N = 25$	ANCA negative, anti-GBM positive $N = 28$
>50% crescents, %	44	38	62	93
Mean % crescents[a]	48 ± 29	46 ± 30	67 ± 32	84 ± 21
Glomerular necrosis[b]	1.2+	1.8+	2.1+	2.1+
Glomerular sclerosis[b]	1.7+	1.3+	1.2+	1.1+
Age	62 ± 15	54 ± 18	68 ± 13	41 ± 21
Male:female	1.0:0.9	1.0:0.7	1.0:1.1	1.0:0.9
Black:White[c]	1.0:6.1	1.0:23.8	1.0:9.0	1.0:4.9
Creatinine	6.9 ± 7.7	5.6 ± 4.6	9.6 ± 5.3	10.0 ± 9.1
Anti-GBM titer	<20	<20	350.5	579.7
ANCA titer	86.0 ± 22	92.9 ± 39	72.3 ± 25	<20

[a] Mean % crescents when crescents are present
[b] Mean of scores of 0 to 4+ with 0 none and 4+ severe
[c] Approximate black:white ratio in the entire renal biopsy population is 1:3

patients with pauci-immune crescentic glomerulonephritis had MPO-ANCA or PR3-ANCA detectable by enzyme-linked immunosorbent assay (ELISA) (unpublished data). Conversely, 87% of patients with renal biopsy findings consistent with anti-GBM disease had detectable antibodies to the noncollagenous domain of type IV collagen (anti-NC1). Some of the 13% of patients with linear GBM IgG but negative assay for anti-NC1 might have had antibodies directed against other antigens in the GBM, such as entactin [16, 19]. The small minority of patients with pauci-immune crescentic glomerulonephritis who do not have detectable MPO-ANCA or PR3-ANCA may have other etiologic agents that produce the same final common pathway of glomerular vascular injury, for example, ANCA with specificities that are not detected by current assays.

The patient under discussion today had both anti-GBM and ANCA antibodies. This is a well-recognized concurrence that occurs in about one-fourth to one-third of patients with anti-GBM antibodies and in a much smaller proportion of patients with ANCA (Table 4) [20–31]. ANCA can be detected before or after anti-GBM antibodies. Anti-GBM antibodies are much more often accompanied by MPO-ANCA than by PR3-ANCA. The anti-GBM and ANCA are two separate populations of antibodies rather than one cross-reacting population [26]. The anti-GBM antibodies that occur with ANCA have the same specificity for the noncollagenous domain of type IV collagen as do anti-GBM antibodies that occur without ANCA [28]. The concurrence of ANCA and anti-GBM antibodies is too frequent to be a coincidence; however, the cause is unknown.

Today's patient had a myeloproliferative disorder and had received a variety of drugs prior to developing ANCA and anti-GBM antibodies. Certain drug exposures are known to induce multiple autoantibodies, including ANCA [6]. For example, hydralazine and propylthiouracil can induce ANCA and pauci-immune crescentic glomerulonephritis [6, 32, 33]. Our patient today received hydroxyurea for a myeloproliferative disorder. Although hydroxyurea is molecularly related to hydralazine, I am not aware of previous reports of hydroxyurea inducing ANCA. The association between myeloproliferative disorders and the development of glomerulonephritis, small vessel vasculitis, and ANCA is poorly defined [34–36]. The link has been observed primarily with myelodysplastic disease or overt myeloid leukemia rather than with the type of myeloproliferative disease present in the patient we are discussing.

Natural history, treatment, and outcome

Current therapy and supportive management have dramatically improved the outcome of rapidly progressive crescentic glomerulonephritis. Still, there is substantial need for further improvement. The prognosis and appropriate treatment for crescentic glomerulonephritis vary depending on the immunopathologic category and disease severity at the time treatment is begun. Anti-GBM and ANCA glomerulonephritis are so aggressive that even patients with little or no crescent formation should be considered for substantial immunosuppressive treatment, especially if pulmonary involvement is present. On the other hand, some categories of immune complex glomerulonephritis, such as IgA nephropathy and acute postinfectious glomerulonephritis, do not necessarily warrant extensive immunosuppressive therapy unless numerous active crescents are present.

A strong predictor of outcome for all types of crescentic glomerulonephritis is the severity of renal insufficiency at the time treatment is begun [6, 37–39]. The pathologic severity, activity, and chronicity of glomerular and tubulointerstitial disease can help refine the prognosis. For example, in patients with ANCA crescentic glomerulonephritis, a higher proportion of histologically

unaffected glomeruli correlates with increased renal survival [40]. The serologic profile correlates in general with presentation and outcomes in patients with ANCA, anti-GBM antibodies, or both. Patients who are anti-GBM positive and ANCA negative have more severe disease at diagnosis (Table 4) and worse outcome [23] than do patients with positive ANCA and negative anti-GBM. Counterintuitively, patients with both anti-GBM and ANCA have less extensive crescent formation (Table 4) and better prognosis [23] than do patients who have anti-GBM without ANCA. Compared to patients with PR3-ANCA, patients with MPO-ANCA have more "chronicity" and less "activity" (pathologically) at the time of diagnosis (Table 4) [41] and have better long-term renal survival [9, 16]. These average differences are not great enough to significantly affect treatment or prognosis in individual patients.

The standard treatment for anti-GBM glomerulonephritis, ANCA glomerulonephritis, and severe crescentic immune complex glomerulonephritis (for example, crescentic lupus nephritis) is high-dose corticosteroids and cytotoxic immunosuppressive drugs [3, 6, 38, 39]. Plasmapheresis is added for anti-GBM glomerulonephritis and for ANCA glomerulonephritis that is accompanied by pulmonary hemorrhage. Levy et al [38] found that the 1-year patient and renal survival rates for anti-GBM glomerulonephritis are 100% and 95%, respectively, if immunosuppression and plasma exchange are begun when the serum creatinine is less than 5.7 mg/dL. If the serum creatinine is 5.7 mg/dL or higher, the 1-year patient and renal survival rates are 83% and 82%, respectively, if dialysis is not necessary initially, but only 65% and 8%, respectively, if dialysis is required.

The exact regimen of immunosuppression for ANCA-associated crescentic glomerulonephritis continues to be refined [6, 39]. Of particular note is the well-organized prospective study of an array of treatment regimens for ANCA glomerulonephritis that is being carried out by the European Vasculitis Study Group [6, 39]. Cyclophosphamide remains the drug of choice for induction of remission. Current immunosuppressive regimens that combine high-dose corticosteroids and cyclophosphamide induce remission in more than 90% of patients [39]. Approximately one-fourth to one-third of patients experience a recurrence within several years. The need for maintenance immunosuppression therapy raises the problem of adequate immunosuppression versus toxicity. Cyclophosphamide has been used extensively in the past, but other remission-maintenance agents being evaluated include azathioprine and mycophenolate mofetil [39]. The goal should be to reduce toxicity while maintaining a recurrence rate of less than 20% at 2 years. Additional therapeutic agents that might prove valuable in the treatment of ANCA-associated glomerulonephritis as well as in other forms of crescentic glomerulonephritis include leflunomide, deoxyspergualin, tumor necrosis factor (TNF) blockade, calcineurin inhibitors, and antibodies against T cells. A better understanding of the pathophysiology of crescentic glomerulonephritis could suggest additional therapeutic options.

Can the pathophysiology of crescent formation offer hints for better treatment?

The cellular and molecular mechanisms that lead to crescent formation are incompletely known. As noted earlier, multiple etiologies and pathogenetic mechanisms can initiate crescent formation, including immune complexes, anti-GBM antibodies or T cells, and ANCA [41]. All these initiating mechanisms might lead to a final common pathway of injury that involves extensive disruption of glomerular capillary walls. This raises the possibility that a critical step in this common pathway might be amenable to treatment that would be effective in multiple kinds of crescentic glomerulonephritides.

Glomerular immune-complex localization appears to be less effective at inducing crescent formation than are ANCA or anti-GBM antibodies (Table 3). When immune-complex glomerulonephritis has crescents, immune complexes usually are identifiable in subendothelial locations, where they are in close proximity to humoral and cellular inflammatory mediator systems in the blood. For example, electron microscopy demonstrates that patients who have lupus nephritis or IgA nephropathy with crescents frequently have subendothelial immune-complex deposits, whereas patients with only mild glomerular inflammation without crescents typically have exclusively mesangial immune-complex deposits [4]. Immune complexes in the subendothelial zone are in an opportune location to attract and activate neutrophils and monocytes, which are then in a prime location to cause maximal glomerular capillary wall injury.

Well over 3 decades ago, Lerner, Glassock, and Dixon established that anti-GBM antibodies cause glomerulonephritis by showing that anti-GBM antibodies from humans cause glomerulonephritis when injected into monkeys [42]. Anti-GBM antibodies have specificity for the C-terminal noncollagenous globular domain of the $\alpha 3$ chain of type IV collagen [43]. A variety of animal models of glomerulonephritis are induced by heterologous or autologous anti-GBM antibodies, and several models support a role for anti-GBM T lymphocytes [44]. Two interesting studies by Wu et al [45, 46] provide some of the strongest evidence that anti-GBM T lymphocytes cause glomerulonephritis. This group induced glomerulonephritis both by active immunization with an antigen that elicited a predominantly T-cell response [45] and by passive transfer of T lymphocytes specific for rat recombinant noncollagenous domain of collagen IV $\alpha 3$ chain [46].

In vitro experiments have demonstrated that ANCA IgG can activate cytokine-primed neutrophils by interacting with MPO or PR3 at the surface of the cells [6, 47]. Neutrophils that have been activated by ANCA release lytic proteinases, toxic oxygen products, and inflammatory cytokines, and these neutrophils can adhere to and kill endothelial cells in culture [6, 47]. This sequence of events occurring in vivo could cause glomerulonephritis and small-vessel vasculitis. A mouse model of MPO-ANCA glomerulonephritis confirms that MPO-ANCA can cause pauci-immune glomerulonephritis with necrosis and crescents, a lesion that closely resembles human ANCA glomerulonephritis [48]. This model is induced by injecting anti-MPO IgG produced in MPO knockout mice into immune-deficient mice that have no functioning B or T lymphocytes of their own. The recipient mice develop pauci-immune focal necrotizing and crescentic glomerulonephritis, pulmonary capillaritis, and necrotizing arteritis within 1 week. This model shows that ANCA IgG can cause glomerulonephritis and small-vessel vasculitis in the absence of antigen-specific T lymphocytes. The anti-MPO IgG also causes pauci-immune focal necrotizing and crescentic glomerulonephritis and systemic vasculitis in immune-competent wild-type mice [48].

How do immune complexes, anti-GBM antibodies or T lymphocytes, and ANCA initiate the inflammatory glomerular injury that culminates in crescent formation? In 1956, Rich at Johns Hopkins proposed that crescents are produced by the proliferation of glomerular epithelial cells in coagulated blood that has spilled into Bowman's space [49]. This concept is in accord with later observations that crescents contain fibrin and that interference with fibrin formation in experimental animal models of crescentic glomerulonephritis eliminates or reduces crescent formation [4, 44, 49– 54]. The fibrin is derived from plasma coagulation factors that spill into Bowman's space through breaks in glomerular capillaries. Scanning electron microscopy shows numerous irregular perforations of GBMs in specimens with crescentic glomerulonephritis [55]. The coagulation factors form fibrin after they come in contact with tissue factor and other thrombogenic materials in Bowman's space [56–57]. Persistence of fibrin in Bowman's space is facilitated by plasminogen activator inhibitor (PAI-1) [53, 57].

Activated coagulation factors and inflammatory cytokines that enter Bowman's space in crescentic glomerulonephritis cause endogenous glomerular cells and infiltrating leukocytes to release many cytokines that stimulate the influx of macrophages and the proliferation of epithelial cells; this process leads to crescent formation [44, 58]. Although breaks in GBMs and fibrin in Bowman's space appear to provide a strong impetus to crescent formation, intense inflammation within glomeruli with intact basement membranes might be capable of inciting crescent formation even without basement membrane disruption [59].

The cells in crescents are predominantly epithelial cells and macrophages [59–63]. The relative proportion of epithelial cells to macrophages varies, depending on the age of the crescent and the integrity of Bowman's capsule [59–63]. Epithelial cells predominate when Bowman's capsule is intact, whereas macrophages predominate when Bowman's capsule is disrupted [63]. Rupture of Bowman's capsule allows monocytes and macrophages to enter not only from the capillary blood but also from the periglomerular interstitium. Periglomerular inflammation, including granulomatous inflammation with multinucleated giant cells, seen in the patient under discussion, is most common when Bowman's capsule is ruptured [64]. Periglomerular granulomatous inflammation is not specific because it can be seen with any type of severe necrotizing glomerulonephritis, including both anti-GBM disease and any type of ANCA disease [4, 64]. Within 1 week or so, cellular elements in crescents begin to disappear through apoptosis [65], and collagen accumulates as a result of synthesis by epithelial cells [66] and fibroblasts that infiltrate through breaks in Bowman's capsule [67]. The transition from cellular to fibrotic crescent might be orchestrated, at least in part, by transforming growth factor-beta (TGF-β), which inhibits epithelial proliferation and promotes fibrosis [68].

As noted earlier, the glomerular injury that appears most effective at initiating crescent formation is rupture of capillary basement membranes, which allows plasma and inflammatory mediators into Bowman's space. This influx can only occur in the presence of lysis of the GBM. Type IV collagen, laminin, fibronectin, and proteoglycans are major components of the GBM. Thus, agents that lyse these proteins and are released during glomerular inflammation are likely important participants in the induction of crescentic glomerulonephritis. Prime candidates include serine proteinases and matrix metalloproteinases (MMPs) that can degrade type IV collagen, laminin, fibronectin, and proteoglycans [69, 70]. Activated neutrophils and monocytes, which are ubiquitous in early crescentic glomerulonephritis of all types, release serine proteinases and MMPs. Perturbed glomerular endothelial, epithelial, and mesangial cells also release MMPs.

Most proteinases are secreted as pro-enzymes that require molecular modification to become active. Notable exceptions are the major serine proteinases (elastase, PR3, and cathepsin G) that are stored in active form within cytoplasmic granules of neutrophils and monocytes, and which are released at sites of leukocyte activation [69, 70]. These serine proteinases can degrade type IV collagen, laminin, fibronectin, and proteoglycans (including heparin sulfate). They all are highly cationic and active at neutral pH and thus can attach to and degrade basement membranes at plasma pH. Elastase might be

the most important proteinase in basement membrane lysis. It also has many inflammatory effects, such as activation of cytokines and induction of endothelial and epithelial cells to secrete cytokines [69]. Elastase and PR3 also kill cells [71], and thus they can destroy not only the capillary basement membranes but also glomerular endothelial and epithelial cells.

The destructive potential of serine proteinases is reflected in the fact that approximately 10% of plasma proteins are serine proteinase inhibitors, such as α1-proteinase inhibitor. Serine proteinases also are inhibited by α_2-macroglobulin, which is a broad-spectrum antiproteinase that also inhibits cysteine proteinases, aspartic proteinases, and MMPs. At sites of inflammatory injury, serine proteinases are protected from neutralization by antiproteinases by multiple mechanisms, including neutralization of antiproteinases by oxidants (especially hypochlorous acid generated by leukocyte respiratory bursts), high concentration of proteinases and exclusion of antiproteinases in the microenvironment between leukocytes and tissue (for example, between leukocytes and GBM), and resistance to inhibition when bound to cell surfaces (for example, leukocyte cell membranes) or tissue (for example, GBM). Elastase that is bound to the surface membrane of neutrophils and monocytes is not only particularly effective at lysis of type IV collagen but also is protected from inactivation by proteinase inhibitors [69, 70].

Matrix metalloproteinases are produced by leukocytes and many other cell types. Some, but not all, MMPs can degrade type IV collagen, laminin, fibronectin, and proteoglycans. Synthesis of MMPs in leukocytes and endogenous glomerular cells is up-regulated after stimulation by inflammatory mediators [69, 70]. However, MMP-8 (collagenase) and MMP-9 (gelatinase) are exceptions because they are synthesized in advance and stored in neutrophil and monocyte granules for rapid release during acute inflammation. As monocytes transform into macrophages, they shift from releasing predominantly serine proteinases to releasing predominantly MMPs [69]. The MMPs are released as proenzymes that must be activated by serine proteinases, oxidants (for example, hypochlorous acid), or active MMPs. Membrane-type MMPs (MT-MMPs) appear to be particularly important for the activation of some pro-MMPs. MMPs are inhibited by α_2-macroglobulin and by tissue inhibitors of MMPs (TIMPs). The inhibitory activity of TIMPs can be blocked by serine proteinases, such as elastase [70].

The respiratory burst of activated neutrophils and monocytes generates oxidants, such as hypochlorous acid, that can synergize with proteinases to lyse the GBM [69, 70]. Stimulated glomerular epithelial cells and mesangial cells also can produce oxidants. Oxidants shield serine proteinases from neutralization by serine proteinase inhibitors and also activate latent metalloproteinase pro-enzymes.

Thus, oxidants, serine proteinases, and metalloproteinases together create a local milieu that can kill glomerular cells and dissolve GBMs. This toxic and lytic microenvironment is very localized, however, because beyond the oxidant shield the serine proteinases are not protected from neutralization and the MMP pro-enzymes are not activated.

Using in vitro experiments, Donavan et al have documented the ability of elastase to degrade GBM, as well as the ability of oxidants to abolish the inhibitory affect of alpha 1-antiproteinase on elastase-mediated GBM degradation [72]. The in vivo relevance of these in vitro studies is supported by the presence of elastase [73] and PR3 [74] at sites of glomerular necrosis in crescentic glomerulonephritis. Further, MMPs, including MT-MMPs, have been identified in glomerular crescents [75].

A number of experimental animal models indicate that T lymphocytes play an important role in crescent formation [44–46]. If this is the case, T lymphocytes must have a direct or indirect mechanism for degrading GBMs. T lymphocytes can degrade collagen directly through the production of MMPs [76]. Granzymes released by T lymphocytes are serine proteinases that are cytotoxic and can lyse proteoglycans; however, granzymes alone are not effective at lysing GBM because they do not degrade collagen or laminin [69]. Activated T lymphocytes also can cause GBM degradation indirectly by stimulating MMP production by endogenous glomerular cells and by inducing release of serine proteinases and MMPs by neutrophils and monocytes. This latter mechanism is the most likely means for T lymphocytes to cause substantial matrix lysis.

Animal data addressing the therapeutic efficacy of recombinant or synthetic proteinases in glomerulonephritis are limited. In a study of immune-complex–mediated glomerulonephritis in mice, intraperitoneal administration of a synthetic serine proteinase inhibitor markedly reduced glomerular necrosis even though immune-complex deposition and endocapillary hypercellularity were not reduced [77]. This at least supports the possibility that proteinase inhibitors could be useful in the treatment of necrotizing glomerulonephritis.

CONCLUSION

A great deal of progress has been made in our understanding and treatment of crescentic glomerulonephritis since the times of Volhard and Fahr, and Ellis. This category of glomerulonephritis, which was almost always fatal, now can be brought into sustained remission in many patients. However, despite the steady incremental improvements in the treatment of crescentic glomerulonephritis over the past 35 years, current toxic immunosuppressive

regimens are far from ideal. As our knowledge of the pathophysiology of crescentic glomerulonephritis improves, I am optimistic that a critical pathogenetic step will be recognized and will be effectively targeted by less-toxic therapeutic agents.

QUESTIONS AND ANSWERS

Dr. John T. Harrington *(Dean, Tufts University School of Medicine, Boston, Massachusetts, USA)*: Let me begin by asking a clinical question. Your second table showed a substantial difference in anti-GBM disease between African Americans and whites. What genetic differences might account for this finding?

Dr. Jennette: I do not think a specific explanation for this difference between African Americans and whites has been found. However, it is in line with other observations of anti-GBM disease that clearly show a genetic predisposition. There are human leukocyte antigen (HLA) phenotypes that are aligned with greater susceptibility for anti-GBM disease. Fisher et al have clearly shown in humans and experimental models evidence for genetic susceptibility to anti-GBM disease [78]. This observation is in accord with the concept that cell-mediated immunity might play an important role in anti-GBM disease in humans as well as in animals because an HLA-association is consistent with a requirement for a particular kind of antigen recognition capability and antigen display capability. This capability could make certain patients susceptible to anti-GBM disease. It may be that African Americans lack this antigen presentation capability. To my knowledge, it has not been looked at in detail.

Dr. Harrington: In a recent *New England Journal of Medicine* review, Schwartz discussed the observation that knockout of *T-bet*, the gene for the transcription factor that induces helper T cells to differentiate into Th1 cells, altered the distribution of T-helper 1 and 2 cells [79]. Have any studies looked at the subtypes of T-helper cells within the glomerular lesions?

Dr. Jennette: There have been a number of studies, most of which have supported the importance of Th1 cells [80]. In fact, in the experimental models by Wu et al [46], the injection of T-helper 1 cells specific for GBM caused crescentic glomerulonephritis in mice. This finding supports the role for cell-mediated immunity in crescentic glomerulonephritis. In all inflammation that progresses from an acute to a chronic phase, an infiltration of lymphocytes orchestrates the inflammatory process as well as healing or scarring if required. However, T cell participation at the site of inflammation does not necessarily mean that these cells were involved in initiating the inflammation. In some types of glomerulonephritis, immunohistochemical evaluation reveals a predominance of T cells and macrophages at some point in time, but this does not prove that an antigen-specific T cell response initiated the event. It means that by the time we biopsied and looked at the tissue, it contained mainly T cells and macrophages. For example, in the model of ANCA disease caused by injection of anti-MPO IgG, even though the Rag 2 mice do not have T cell antigen recognition capability, after the lesion evolves for 2 weeks, lymphocytes appear at the site of inflammation. In this model, anti-MPO IgG causes crescentic glomerulonephritis in the absence of T lymphocytes [48]. Thus, in some models, Th1 cells seem to be important, and in others they are not.

Dr. Annamaria Kausz *(Division of Nephrology, New England Medical Center, Boston, Massachusetts, USA)*: You spoke of the differences in outcomes of patients with ANCA versus anti-GBM versus both. If you believe that the antibody itself is pathogenetic, why is the outcome of those with both ANCA and anti-GBM antibodies better than that of those with only anti-GBM?

Dr. Jennette: It is difficult for me to understand why that is the case. To my knowledge, only one study has documented that the prognosis is better with both [23]. However, our own observations provide additional support because we see an intermediate degree of injury, not a greater degree when ANCA and anti-GBM are both present (Table 4). The titer of the antibodies does not seem to be a factor. In our experience, both the anti-GBM and the ANCA titers are a little bit lower when they are both together (Table 4), but other groups have not seen any difference.

I must say that I do not understand the dual positivity at all. It would be easier to hypothesize what is going on if one came consistently before the other. If ANCA disease always came first and anti-GBM second, then you could say it was a disruption of the GBM by the ANCA disease that has engendered the anti-GBM response. But sometimes it is the other way around. I do not understand that association at all.

Dr. Geetha Narayan *(Division of Nephrology, St. Elizabeth's Medical Center, Brighton, Massachusetts, USA)*: It was announced at the last American Society of Nephrology (ASN) meeting that a European study group had updated their studies of treatment for ANCA disease. They were asked how long they were planning to continue maintenance therapy in a specific patient and hinted that it might be indefinite. Based on your understanding of the pathophysiologic mechanism and the possible history, do you think that this is a disease that is an unrelenting, chronic process that can be controlled with indefinite therapy?

Dr. Jennette: We are probably going to have to expunge this answer because I am a pathologist! There is the empiric response, and then there is the hypothetical response based on what we think we know about the pathogenesis. The empiric approach is to titrate the

maintenance of remission against the toxicity from the maintenance therapy. As I said, if we give extensive immunosuppressive agents, there is still an approximately 20% relapse within the first 3 years or so, and sometimes as much as 50% if we don't provide as much immunosuppression. For example, if you give a standard regimen, you may have a 30% recurrence rate after remission, and you are worried about that 30%. You might want to give more immunosuppressive agents for a longer period to obtain less recurrence. If you do that, it means that 70% of the patients who were not going to have a recurrence will have been given more immunosuppression than they needed to protect the 30% who would have a recurrence. You might want to give maintenance therapy until you reduce the recurrence rate to an acceptable plateau and then give additional re-induction therapy for recurrences.

With respect to the pathogenesis, I am concerned that ANCA disease is a persistent disease. In contrast to anti-GBM, ANCA usually hangs around for a long time. We might need some sort of long-term maintenance therapy for ANCA disease, possibly something that can be directed specifically toward the neutrophils and monocytes.

Dr. Angelo A. Ucci, Jr. *(Division of Pathology, New England Medical Center)*: Your suggestion that we intervene during the early steps in the evolution of this injury is very interesting. My experience in looking at biopsies is that early changes in the glomerulus are not terribly common. I more often see the later stages of injury with epithelial crescents and find areas of necrosis involving the capillary walls. Has that been your experience? Have you thought about ways that we might better ascertain these injuries at an early stage?

Dr. Jennette: I think that earlier diagnosis will be obtained in the clinical arena. General physicians need to be more aware in their initial contacts with these patients, we need more rapid referral to nephrologists, and diagnosis needs to be faster. Is there room for improvement? Once the diagnosis is made, we generally are successful; most patients used to die of their disease, but now most of them do not. With ANCA disease, in our experience, only 50% of patients have >50% glomeruli involved with crescents. That means that 50% of the patients whom we see have 50% or more of their glomeruli without any crescents yet. Some might have segmental necrosis. As a pathologist, I tend to home in on the most severe lesions when I look at a specimen, and those are the things that hold my attention. The European group has suggested that we pay attention to the glomeruli that are not involved [81]. In fact, the percentage of uninvolved glomeruli is the best prognostic indicator [40], which leads to your point about how much injury exists in the beginning. I think that most patients have a reasonable number of preserved nephrons, and those nephrons are the ones that we want to save. I think that current therapy is directed more at neutrophils than at lymphocytes. I wonder whether the therapy that is most effective is not the therapy that is most immunosuppressive, but the therapy that reduces neutrophil and monocyte production by the bone marrow.

Dr. Andrew S. Levey *(Division of Nephrology, New England Medical Center)*: I am intrigued by your description of the publication of the induction of crescentic glomerulonephritis by lymphocytes against the putative antigen of anti-GBM disease [46]. I imagine that you are trying to raise those kinds of lymphocytes in your MPO knockout mice. Do you have any results to tell us about? In particular, you downplayed the possibility that lymphocytes might injure GBMs. What causes people to develop these autoantibodies and autoreactive lymphocytes?

Dr. Jennette: With respect to the model [48], the first version that we created began with immunization of MPO knockout mice with MPO and then transfer of their splenocytes into the immune-deficient Rag 2 knockout mice. We were using immune-deficient mice because we were transferring the immune system from the MPO knockout mice to these mice. Of course, they adopted it. Within a few days, the anti-MPO titer in the recipient animal reached the same level as in the donor animal. The T cells and B cells take hold in the recipient mice. One feature of that model might be a problem; it depends on your perspective. No matter what set of splenocytes we transferred into the immune-deficient mice, whether it was MPO knockout mice that were immunized with nothing, MPO knockout mice immunized with bovine serum albumin (BSA), or MPO knockout mice immunized with MPO, when we transferred their immune systems into the immune-deficient mice, those mice developed mild immune-complex glomerulonephritis. They all have clinically insignificant mesangial immune-complex localization. Only the mice that receive the transfer of anti-MPO–specific splenocytes develop glomerular disease that is necrotizing and crescent forming and very severe—90% of glomeruli with necrosis and crescents. The next step was to dissect the pathogenetic process—transfer just anti-MPO antibodies, T lymphocytes, and both. The easier of those two challenges was to transfer just antibodies. We took serum from the knockout mice that were immunized with MPO and isolated the IgG. In the first experiment, we thought we were just going to determine the titer based on passive transfer of IgG so we could decide what dose to give. We never thought that in the first experiment all five mice that received anti-MPO IgG would get necrotizing glomerulonephritis [48]. As yet, we have not done experiments involving transfer of T lymphocytes or T lymphtocytes plus anti-MPO antibodies.

Dr. Kausz: You mentioned the GBM and role of

proteases, and how the proteases might be protected from proteinase inhibitors by the microenvironment. You alluded to an experiment in which proteinase inhibitors injected into mice protected them from necrotizing glomerulonephritis [77]. Is this just a situation in which you overwhelm the microprotection?

Dr. Jennette: The problem is delivery of antiproteinase to the site of the inflammation. Pharmaceutical companies are looking for antiproteinases as therapeutic agents for asthma, arthritis, and other diseases where there is a big market for anti-inflammatory agents. One thing that is suspected, and in some instances documented, is that you need small molecules. The proteinase inhibitor we used was not a protein [77]. The naturally occurring antiproteinases are pretty big molecules—they are proteins. If you could find a nontoxic, small-molecule antiproteinase, it might be an effective treatment. The problem is that there are many biologically important proteinases. You have to be careful to come up with some therapeutic agent that can neutralize the ones that you are concerned with without destroying the others.

Dr. Katrin Uhlig *(Division of Nephrology, New England Medical Center)*: I am curious about the connection between anti-proteinases and ANCA disease. As you know, genetic α1 antitrypsin deficiency might be associated with ANCA-positive glomerulonephritis [81]. Can we learn anything from the National Institutes of Health (NIH) studies of systemic α1 antitrypsin augmentation therapy in patients with interstitial lung disease?

Dr. Jennette: I am not familiar with that study, but I think that any other inflammatory states showing benefit from antiproteinase treatment would be worth looking at. There is an antiproteinase in the respiratory tract called secretory leukoproteinase inhibitor (SLPI), which is an unusually small proteinase inhibitor. Some work has been done using SLPI added to the respiratory tract for local anti-inflammatory effects [82]. With lung disease, you are in a better position because you do not have to get the antiproteinase into the circulation. The problem with trying to treat glomerulonephritis or vasculitis with proteinase inhibitors is that you have to put them into the circulation and yet not interrupt important serum cascades of homeostatic proteins such as the coagulation cascade.

Dr. John Gill *(St. Paul's Hospital, Vancouver, British Columbia, Canada)*: Often we learn from the atypical cases. You mentioned a possible role for cell-mediated mechanisms of injury in anti-GBM disease. Could cellular mechanisms also be important in the group of patients with pauci-immune disease who are ANCA-negative?

Dr. Jennette: It is certainly possible. If most ANCA disease is antibody mediated, and now I am more convinced of that than in the past, and the remaining group was cell mediated, you would expect there to be some subtle difference in the pathology, but there is not. I cannot see any difference in the pauci-immune necrotizing glomerulonephritis that is ANCA-negative versus ANCA-positive. However, having said that, I cannot see any pathologic difference at the light microscopic level between ANCA glomerulonephritis and anti-GBM glomerulonephritis. If the latter is shown to be cell-mediated, then I will have to adjust my thinking. I am still holding on to the concept that anti-GBM antibodies are pathogenetic in humans.

Dr. Gill: Would you comment on the prognosis and management of this group of ANCA-negative patients with pauci-immune disease?

Dr. Jennette: There is no clinical or pathologic difference. They can have any of the phenotypes of ANCA-positive disease. They can have a clinical and pathologic expression of Wegener's granulomatosis or microscopic polyangiitis that is identical to what is seen in a patient who is ANCA-positive. Most patients with Churg-Strauss syndrome are ANCA-negative. Some people have contended that this evidence indicates that ANCAs are not pathogenetic. Like all negative data, these data are less powerful than the positive data that support a pathogenetic role for ANCA. I am more confident now that ANCA does cause disease in ANCA-positive patients. An alternative mechanism in ANCA-negative patients might be causing the same pathologic phenotype and the same clinical phenotype. We treat all patients the same whether they have ANCA-positive pauci-immune glomerulonephritis or ANCA-negative pauci-immune glomerulonephritis.

Dr. Vaidyanatha Balakrishnan *(Division of Nephrology, New England Medical Center)*: My question relates to the role of antioxidants. Given the ability of reactive oxygen species to inactivate proteinases, have you any experience with anti-oxidants in the early phase of animal studies?

Dr. Jennette: Even before we studied the mice with immune-complex disease with the antiproteinase inhibitors back in the 1980s [77], we thought about using anti-oxidants in mice with glomerulonephritis. We did use superoxide dismutase in immune-complex models, and we did see a reduction in severity of the disease, but this was published only as an abstract (abstract; Jennette JC, *Fed Proc* 41:325, 1982). A number of other studies in experimental models have shown that anti-oxidant therapy can reduce inflammation [83]. I think anti-oxidants are another possibility. It may be that we need to look for are additive effects—anti-oxidants plus antiproteinases—because of the additive pathogenetic interaction of oxidants and proteinases. Some studies have found a reduction in the level of neutrophil activity in patients with ANCA disease who were given anti-oxidant vitamin therapy [84]. I do not recall any correlation with any clinical parameters, but at least the first step has been

taken to determine the effects of anti-oxidants on neutrophils in patients with ANCA disease.

Dr. Harrington: What is the current approach at Chapel Hill in patients who have achieved a remission from crescentic glomerular disease but who subsequently have rising serologies without evidence of clinical activity?

Dr. Jennette: There is a heightened sense of concern and alertness for further evidence of disease recurrence. The approach is not to treat the titer. The best evidence supporting treatment based on titer is the work by Cohen Tervaert et al in Europe [85]. They showed that if treatment begins when the titer begins to rise, patients have less recurrence of disease manifestations. In the long run, patients treated on the basis of titer received less total immunosuppression because they had less recurrence than the group who were not treated on the basis of ANCA titer who then required more immunosuppression to bring their relapses under control. A number of studies, however, have shown very little correlation between ANCA titers and recurrence. We are concerned and vigilant when we see the ANCA titer rising, but we do not treat on the basis of this finding alone.

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REFERENCES

1. Volhard F, Fahr T: Die brightsche nierenkrankheit: klink, pathologic und atlas. Berlin, Springer, 1914, p 300
2. Ellis A: Natural history of Bright's disease: Clinical, histological and experimental observations. *Lancet* 1:1–7, 1942
3. Couser WG: Rapidly progressive glomerulonephritis: classification, pathogenetic mechanisms, and therapy. *Am J Kidney Dis* 11:449–464, 1988
4. Jennette JC: Crescentic glomerulonephritis, in *Heptinstall's Pathology of the Kidney* (5th ed), edited by Jennette JC, Olson JL, Schwartz MM, Silva FG, Philadelphia, Lippincott-Raven, 1998, pp 625–656
5. Harris AA, Falk RJ, Jennette JC: Crescentic glomerulonephritis with a paucity of glomerular immunoglobulin localization. *Am J Kidney Dis* 32:179–184, 1998
6. Savage COS: ANCA-associated renal vasculitis. *Kidney Int* 60: 1614–1627, 2001
7. Jennette JC, Falk RJ: Small vessel vasculitis. *N Engl J Med* 337: 1512–1523, 1997
8. Savige J, Gillis D, Davies D, *et al*: International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol* 111:507–513, 1999
9. Franssen CFM, Stegeman CA, Kallenberg CGM, *et al*: Antiproteinase 3- and antimyeloperoxidase-associated vasculitis. *Kidney Int* 57:2195–2206, 2000
10. Wilson CB, Dixon FJ: Anti-glomerular basement membrane antibody-induced glomerulonephritis. *Kidney Int* 3:74–89, 1973
11. Savage CO, Pusey CD, Bowman C, *et al*: Antiglomerular basement membrane antibody mediated disease in the British Isles 1980–4. *Br Med J (Clinical Res Ed)* 292:301–304, 1986
12. Churchill DN, Fine A, Gault MH: Association between hydrocarbon exposure and glomerulonephritis. An appraisal of the evidence. *Nephron* 33:169–172, 1983
13. Bonzel KE, Muller-Wiefel DE, Ruder H, *et al*: Anti-glomerular basement membrane antibody-mediated glomerulonephritis due to glue sniffing. *Eur J Pedi*atr 146:296–300, 1987
14. Evans MD, Pryor WA: Cigarette smoking, emphysema, and damage to alpha 1-proteinase inhibitor. *Am J Physiol* 266(6 Pt 1):L593–L611, 1994
15. Niles JL, Bottinger EP, Saurina GR, *et al*: The syndrome of lung hemorrhage and nephritis is usually an ANCA-associated condition. *Arch Intern Med* 156:440–445, 1996
16. Saxena R, Bygren P, Rasmussen N, Wieslander J: Circulating autoantibodies in patients with extracapillary glomerulonephritis. *Nephrol Dial Transplant* 6:389–397, 1991
17. Hagen EC, Daha MR, Hermans J, *et al*: Diagnostic value of standardized assays for anti-neutrophil cytoplasmic autoantibodies in idiopathic systemic vasculitis. *Kidney Int* 53:743–753, 1998
18. Lim LCL, Taylor JG, III, Schmitz JL, *et al*: Diagnostic usefulness of antineutrophil cytoplasmic autoantibody serology: Comparative evaluation of commercial indirect fluorescent antibody kits and enzyme immunoassay kits. *Am J Clin Pathol* 111:363–369, 1999
19. Saxena R, Bygren P, Arvastson B, Wieslander J: Circulating autoantibodies as serological markers in the differential diagnosis of pulmonary renal syndrome. *J Intern Med* 238:143–152, 1995
20. O'Donoghue DJ, Short CD, Brenchley PE, *et al*: Sequential development of systemic vasculitis with anti-neutrophil cytoplasmic antibodies complicating anti-glomerular basement membrane disease. *Clin Nephrol* 32:251–255, 1989
21. Jayne DR, Marshall PD, Jones SJ, Lockwood CM: Autoantibodies to GBM and neutrophil cytoplasm in rapidly progressive glomerulonephritis. *Kidney Int* 37:965–970, 1990
22. Vanhille P, Noel LH, Reumaux D, *et al*: Late emergence of systemic vasculitis with anti-neutrophil cytoplasmic antibodies in a dialyzed patient with anti-glomerular basement glomerulonephritis. *Clin Nephrol* 33:257–258, 1990
23. Bosch X, Mirapeix E, Font J, *et al*: Prognostic implication of anti-neutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in anti-glomerular basement membrane disease. *Clin Nephrol* 36:107–113, 1991
24. Weber MF, Andrassy K, Pullig O, *et al*: Antineutrophil-cytoplasmic antibodies and antiglomerular basement membrane antibodies in Goodpasture's syndrome and in Wegener's granulomatosis. *J Am Soc Nephrol* 2:1227–1234, 1992
25. Wanten GJ, Koolen MI, Harthoorn-Lasthuizen EJ, *et al*: Antineutrophil cytoplasmic autoantibodies with myeloperoxidase specificity in a patient with anti-glomerular basement membrane disease. *Neth J Med* 47:25–29, 1995
26. Short AK, Esnault VL, Lockwood CM: Anti-neutrophil cytoplasm antibodies and anti-glomerular basement membrane antibodies: Two coexisting distinct autoreactivities detectable in patients with rapidly progressive glomerulonephritis. *Am J Kidney Dis* 26:439–445, 1995
27. Kalluri R, Meyers K, Mogyorosi A, *et al*: Goodpasture syndrome involving overlap with Wegener's granulomatosis and anti-glomerular basement membrane disease. *J Am Soc Nephrol* 8:1795–1800, 1997
28. Hellmark T, Niles JL, Collins AB, *et al*: Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol* 8:376–385, 1997
29. Meisels IS, Stillman IE, Kuhlik AB: Anti-glomerular basement membrane disease and dual positivity for antineutrophil cytoplasmic antibody in a patient with membranous nephropathy. *Am J Kidney Dis* 32:646–648, 1998
30. Verburgh CA, Bruijn JA, Daha MR, van Es LA: Sequential development of anti-GBM nephritis and ANCA-associated pauci-immune glomerulonephritis. *Am J Kidney Dis* 34:344–348, 1999
31. Peces R, Rodriguez M, Pobes A, Seco M: Sequential development of pulmonary hemorrhage with MPO-ANCA complicating anti-glomerular basement membrane antibody-mediated glomerulonephritis. *Am J Kidney Dis* 35:954–957, 2000
32. D'Cruz D, Chesser AM, Lightowler C, *et al*: Antineutrophil cytoplasmic antibody-positive crescentic glomerulonephritis associated with anti-thyroid drug treatment. *Br J Rheumatol* 34:1090–1091, 1995
33. Nassberger L, Johansson AC, Bjorck S, Sjoholm AG: Antibodies to neutrophil granulocyte myeloperoxidase and elastase: Auto-

immune responses in glomerulonephritis due to hydralazine treatment. *J Intern Med* 229:261–265, 1991
34. Savige JA, Chang L, Smith CL, Duggan JC: Myelodysplasia, vasculitis and anti-neutrophil cytoplasm antibodies. *Leuk Lymphoma* 9:49–54, 1993
35. Komatsuda A, Miura I, Ohtani H, *et al*: Crescentic glomerulonephritis accompanied by myeloperoxidase-antineutrophil cytoplasmic antibodies in a patient having myelodysplastic syndrome with trisomy 7. *Am J Kidney Dis* 31:336–340, 1998
36. Hamidou MA, Derenne S, Audrain MA, *et al*: Prevalence of rheumatic manifestations and antineutrophil cytoplasmic antibodies in haematological malignancies. A prospective study. *Rheumatology* 39:417–420, 2000
37. Hogan SL, and the Glomerular Disease Collaborative Network: Prognostic markers in patients with ANCA-associated microscopic polyangiitis and glomerulonephritis. *J Am Soc Nephrol* 7:23–32, 1996
38. Levy JB, Turner AN, Rees AJ, Pusey CD: Long-term outcome of anti-glomerular basement membrane antibody disease treated with plasma exchange and immunosuppression. *Ann Intern Med* 134:1033–1042, 2001
39. Jayne D: European Vasculitis Study Group: Update on the European Vasculitis Study Group trials. *Curr Opin Rheumatol* 13:48–55, 2001
40. Bajema IM, Hagen EC, Hermans J, *et al*: Kidney biopsy as a predictor for renal outcome in ANCA-associated necrotizing glomerulonephritis. *Kidney Int* 56:1751–1758, 1999
41. Hauer HA, Bajema IM, van Houwelingen HC, *et al*: Renal histology in ANCA-associated vasculitis: Differences between diagnostic and serologic subgroups. *Kidney Int* 61:80–89, 2002
42. Lerner RA, Glassock RJ, Dixon FJ: The role of anti-glomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J Exp Med* 126:989–1004, 1967
43. Kalluri R, Sun MJ, Hudson BG, Neilson EG: The Goodpasture autoantigen. Structural delineation of two immunologically privileged epitopes on alpha3(IV) chain of type IV collagen. *J Biol Chem* 271:9062–9068, 1996
44. Tipping PG, Kitching AR, Cunningham MA, Holdsworth SR: Immunopathogenesis of crescentic glomerulonephritis. *Curr Opin Nephrol Hypertens* 8:281–286, 1999
45. Wu J, Hicks J, Ou C, *et al*: Glomerulonephritis induced by recombinant collagen IVα3 chain noncollagen domain 1 is not associated with glomerular basement membrane antibody: A potential T cell-mediated mechanism. *J Immunol* 167:2388–2395, 2001
46. Wu J, Hicks J, Borillo J, *et al*: CD4 T cells specific for glomerular basement membrane antigen mediate glomerulonephritis. *J Clin Invest* 109:517–524, 2002
47. Jennette JC, Falk RJ: Pathogenesis of the vascular and glomerular damage in ANCA-positive vasculitis. *Nephrol Dial Transplant* 13(Suppl 1):16–20, 1998
48. Xiao H, Heeringa P, Hu P, *et al*: Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase (MPO-ANCA) cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 110:955–963, 2002
49. Rich AR: The pathology and pathogenesis of experimental anaphylactic glomerulonephritis in relation to human acute glomerulonephritis. *Bull Johns Hopkins Hosp* 98:120–151, 1956
50. Vassalli P, McCluskey RT: The pathogenic role of the coagulation process in rabbit Masugi nephritis. *Am J Pathol* 45:653–677, 1964
51. Tipping PG, Thomson NM, Holdsworth SR: A comparison of fibrinolytic and defibrinating agents in established experimental glomerulonephritis. *Br J Exp Pathol* 67:481–491, 1986
52. Zoja C, Corna D, Macconi D, *et al*: Tissue plasminogen activator therapy of rabbit nephrotoxic nephritis. *Lab Invest* 62:34–40, 1990
53. Kitching AR, Holdsworth SR, Ploplis VA, *et al*: Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med* 185:963–968, 1997
54. Drew AF, Tucker HL, Liu H, *et al*: Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice. *Am J Physiol* 281:F1157–F1163, 2001
55. Bonsib SM: Glomerular basement membrane necrosis and crescent organization. *Kidney Int* 33:966–974, 1988
56. Erlich JH, Holdsworth SR, Tipping PG: Tissue factor initiates glomerular fibrin deposition and promotes major histocompatibility complex class II expression in crescentic glomerulonephritis. *Am J Pathol* 150:873–880, 1997
57. Grandaliano G, Gesualdo L, Ranieri E, *et al*: Tissue factor, plasminogen activator inhibitor-1, and thrombin receptor expression in human crescentic glomerulonephritis. *Am J Kidney Dis* 35:726–738, 2000
58. Atkins RC, Nikkolic-Paterson DJ, Song Q, Lan HY: Modulators of crescentic glomerulonephritis. *J Am Soc Nephrol* 7:2271–2278, 1996
59. Le Hir M, Keller C, Eschmann V, *et al*: Podocyte bridges between the tuft and Bowman's capsule: An early event in experimental crescentic glomerulonephritis. *J Am Soc Nephrol* 12:2060–2071, 2001
60. Magil AB: Histogenesis of glomerular crescents. Immunohistochemical demonstration of cytokeratin in crescent cells. *Am J Pathol* 120:222–229, 1985
61. Jennette JC, Hipp CG: The epithelial antigen phenotype of glomerular crescent cells. *Am J Clin Pathol* 86:274–280, 1986
62. Guettier C, Nochy D, Jacquot C, *et al*: Immunohistochemical demonstration of parietal epithelial cells and macrophages in human proliferative extra-capillary lesions. *Virchows Arch* 409:739–748, 1986
63. Boucher A, Droz D, Adafer E, Noel LH: Relationship between the integrity of Bowman's capsule and the composition of cellular crescents in human crescentic glomerulonephritis. *Lab Invest* 56:526–533, 1987
64. Bajema IM, Hagen EC, Ferrario F, *et al*: Renal granulomas in systemic vasculitis. *Clin Nephrol* 48:16–21, 1997
65. Shimizu A, Masuda Y, Kitamura H, *et al*: Apoptosis in progressive crescentic glomerulonephritis. *Lab Invest* 74:941–951, 1996
66. Gabbert H, Thoenes W: Formation of basement membrane in extracapillary proliferates in rapidly progressive glomerulonephritis. *Virchows Arch* 25:265–269, 1977
67. Yoshioka K, Tohda M, Takemura T, *et al*: Distribution of type I collagen in human kidney diseases in comparison with type III collagen. *J Pathol* 162:141–148, 1990
68. Adler S, Chen X, Eng B: Control of rat glomerular epithelial cell growth in vitro. *Kidney Int* 37:1048–1054, 1990
69. Owen CA, Campbell EJ: The cell biology of leukocyte-mediated proteolysis. *J Leuko Biol* 65:137–150, 1999
70. Witko-Sarsat V, Rieu P, Descamps-Latscha B, *et al*: Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 80:617–653, 2000
71. Yang JJ, Kettritz R, Falk RJ, *et al*: Apoptosis of endothelial cells induced by the neutrophil serine proteases proteinase 3 and elastase. *Am J Pathol* 149:1617–1626, 1996
72. Donavan KL, Davies M, Coles GA, Williams JD: Relative roles of elastase and reactive oxygen species in the degradation of human glomerular basement membranes by intact human neutrophils. *Kidney Int* 45:1555–1561, 1994
73. Oda T, Hotta O, Taguma Y, *et al*: Involvement of neutrophil elastase in crescentic glomerulonephritis. *Hum Pathol* 28:720–728, 1997
74. Bajema IM, Hagen EC, de Heer E, *et al*: Colocalization of ANCA-antigens and fibrinoid necrosis in ANCA-associated vasculitis. *Kidney Int* 60:2025–2030, 2001
75. Hayashi K, Horikoshi S, Osada S, *et al*: Macrophage-derived MT1-MMP and increased MMP-2 activity are associated with glomerular damage in crescentic glomerulonephritis. *J Pathol* 191: 299–305, 2000
76. Esparza J, Vilardell C, Calvo J, *et al*: Fibronectin upregulates gelatinase B (MMP-9) and induces coordinated expression of gelatinase A (MMP-2) and its activator MT1-MMP (MMP-14) by human T lymphocyte cell lines. A process repressed through RAS/MAP kinase signaling pathways. *Blood* 94:2754–2766, 1999
77. Jennette JC, Tidwell RR, Geratz JD, Falk RJ: Amelioration of immune complex mediated glomerulonephritis by synthetic protease inhibitors. *Am J Pathol* 127:499–506, 1987
78. Fisher M, Pusey CD, Vaughan RW, Rees AJ: Susceptibility to anti-glomerular basement membrane disease is strongly associated with HLA-DRB1 genes. *Kidney Int* 51:222–229, 1997

79. Schwartz RS: A new element in the mechanism of asthma. *N Engl J Med* 346:857–858, 2002
80. Kitching AR, Holdsworth SR, Tipping PG: Crescentic glomerulonephritis–a manifestation of a nephritogenic Th1 response? *Histol Histopathol* 15:993–1003, 2000
81. Hubbard RC, Crystal RG: Alpha-1-antitrypsin augmentation therapy for alpha-1-antitrypsin deficiency. *Am J Med* 84:52–62, 1988
82. Tomee JF, Koeter GH, Hiemstra PS, Kauffman HF: Secretory leukoprotease inhibitor: A native antimicrobial protein presenting a new therapeutic option? *Thorax* 53:114–116, 1998
83. Suwannaroj S, Lagoo A, Keisler D, McMurray RW: Antioxidants suppress mortality in the female NZB x NZW F1 mouse model of systemic lupus erythematosus. *Lupus* 10:258–265, 2001
84. Harper L, Nuttall SL, Martin U, Savage CO: Adjuvant treatment of patients with antineutrophil cytoplasmic antibody-associated vasculitis with vitamins E and C reduces superoxide production by neutrophils. *Rheumatology* 41:274–278, 2002
85. Cohen Tervaert JW, Huitema MG, Hene RJ, *et al*: Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 336:709–711, 1990