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Anti-Neutrophil Cytoplasmic Autoantibody-Associated Glomerulonephritis and Vasculitis

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Anti-neutrophil cytoplasmic autoantibodies (ANCA) react with constituents of neutrophil primary granules and monocyte lysosomes. Indirect immunofluorescence microscopy using alcohol-fixed neutrophils demonstrates two ANCA types: one causing cytoplasmic staining (C-ANCA), and a second causing artifactual perinuclear staining (P-ANCA) that frequently has specificity for myeloperoxidase. Using indirect immunofluorescence microscopy (IIFM) and enzyme immunoassays (EIA), sera from over 300 patients with renal disease, with and without systemic vasculitis, were analyzed. Of 76 patients with pauci-immune glomerulonephritis with crescents or necrosis, 87% had ANCA by IIFM (38% of C-ANCA type, 49% of P-ANCA type), and 78% had ANCA by EIA. Of 55 patients with nonlupus immune complex-mediated glomerulonephritis, only 11% had ANCA by IIFM and 5% had ANCA by EIA. Of 24 patients with anti-GBM antibody-mediated glomerulonephritis, none had ANCA. Renal and extrarenal lesions were studied in 81 patients with ANCA-associated glomerulonephritis. These patients formed a pathologic continuum ranging from renal-limited to widespread systemic vascular injury, including patients with primary crescentic glomerulonephritis, Wegener's granulomatosis, and polyarteritis nodosa. In ANCA-positive patients the frequency of C-ANCA and P-ANCA correlated with disease distribution. P-ANCA was most frequent with renal-limited disease and C-ANCA was most frequent when there was lung and sinus involvement. It is proposed that ANCA are not only useful diagnostic markers, but may also be directly involved in a novel pathogenetic mechanism that is a frequent cause of crescentic glomerulonephritis and systemic necrotizing vasculitis. (Am J Pathol 1989, 135:921–930)

The clinical and pathologic diagnosis of systemic necrotizing vasculitis is difficult. A major confounding problem is that different pathogenetic mechanisms can produce structurally similar vascular lesions. Therefore, diagnostic classification systems based solely on morphology are inadequate for optimum categorization. Pathogenetic classification systems have suffered from the problem that a large proportion of vasculitides do not show convincing evidence for mediation by the two most widely recognized mechanisms, ie, immune complex-mediated injury and anti-basement membrane autoantibody-mediated injury. We propose that there is a third major mechanism of vascular injury that involves direct activation of neutrophils and mononuclear phagocytes by anti-neutrophil cytoplasmic autoantibodies (ANCA). If this mechanism exists, activated leukocytes would attack vessel walls, producing injury similar to that caused by immune complex localization or anti-basement membrane antibody binding.

ANCA were first reported by Davies et al[1] in eight patients with hematuria and arthralgias or myalgias. Four of these patients had dyspnea or hemoptysis and two had extensive pulmonary disease identified radiographically. On renal biopsy, all eight patients had segmental necrotizing glomerulonephritis with crescent formation and no immune deposits by immunofluorescence microscopy. In 1984, Hall et al[2] reported on four ANCA-positive patients. All four patients had arthralgias and pulmonary disease. Three of the four had focal necrotizing glomerulonephritis, two had cutaneous vasculitis, and two had gastrointestinal symptoms.[2] In 1985, van der Woude et al[3] demonstrated a high sensitivity of ANCA for active Wegener's granulomatosis and noted that the presence of ANCA correlated with disease activity. The correlation of ANCA titer with disease activity in Wegener's granulomatosis patients was confirmed by Gross et al.[4] Subsequent studies identified ANCA in patients with Wegener's granulomatosis, polyarteritis nodosa, Churg-Strauss syndrome, and pauci-immune necrotizing glomerulonephritis.[5–12]

ANCA react with antigens in the cytoplasm of neutrophils and monocytes. These antigens are in the primary

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Table 1. *The Occurrence of ANCA**

	No.	Positive	IIFM		EIA	
			C-ANCA	P-ANCA	Mean	Positive†
Pauci-immune necrotizing GN	76	66 (87%)	29 (38%)	37 (49%)	75.5	59 (78%)
Anti-GBM-mediated GN	24	0 (0%)	0 (0%)	0 (0%)	19.9	0 (0%)
Immune complex-mediated GN (nonlupus)	55	4 (7%)	0 (0%)	4 (7%)	23.3	3 (5%)

* In patients with biopsy-proven pauci-immune necrotizing GN, anti-GBM antibody-mediated GN and nonlupus immune complex mediated GN.
† A positive EIA level was considered to be a value greater than the mean plus two standard deviations of 83 blood bank control specimens, ie, greater than 42.2.

granules of neutrophils[10] and in the lysosomes of monocytes. All ANCA, however, do not have specificity for the same granule constituents. Because of different specificities, by indirect immunohistology different ANCA types produce different immunostaining patterns on alcohol-fixed neutrophils. Of the two major ANCA types, one (C-ANCA), which appears to have specificity for an approximately 30 kd component of primary granules,[13] produces cytoplasmic staining with central accentuation (Figure 1A). The second type (P-ANCA), usually having myeloperoxidase specificity in patients with renal disease,[10] produces artifactual perinuclear staining of alcohol-fixed neutrophils (Figure 1B) but cytoplasmic staining of formalin-fixed neutrophils (Figure 1C). The type of ANCA present correlates to a degree with the distribution and nature of vascular injury; for example, as will be documented later, patients with Wegener's granulomatosis have a higher frequency of C-ANCA whereas patients with pauci-immune necrotizing glomerulonephritis and no evidence of extrarenal disease have a higher frequency of P-ANCA.[11]

In this article we present data indicating that ANCA are present in the blood of patients with a number of necrotizing vasculitic syndromes that lie within a continuum of ANCA-associated vascular injury. We also give pathologic descriptions of the renal and extrarenal lesions observed in patients with ANCA, which indicate that some of these patients meet the clinicopathologic criteria for polyarteritis nodosa, Wegener's granulomatosis, or pauci-immune ("idiopathic") necrotizing glomerulonephritis, whereas others have clinical and pathologic features that overlap among these syndromes.

We propose that ANCA are not only a marker that will facilitate classification of vasculitides and glomerulonephritides, but also are pathogenetic factors in a previously unrecognized mechanism of immune-mediated tissue injury.

Materials and Methods

Tissue Specimens and Serum Samples

All patients in this study had renal disease with or without evidence for systemic vasculitis. All patients underwent renal biopsy. All renal biopsy specimens were evaluated by the Nephropathology Laboratory of the University of North Carolina at Chapel Hill. Renal biopsies were examined by standard light, immunofluorescence, and electron microscopy techniques.[14] Three different series of patients selected by different criteria were analyzed to determine the following: 1) The association of ANCA with different immunohistopathologic categories of renal disease (more than 300 patients with renal biopsy immunofluorescence microscopy data and ANCA data were analyzed). 2) The frequency of ANCA-associated glomerulonephritis among all patients with renal disease (the renal biopsy findings in 1500 consecutive nontransplant renal biopsies were evaluated). 3) The range of pathologic renal and extrarenal changes in patients with ANCA-associated glomerulonephritis (81 patients with ANCA and glomerulonephritis were evaluated by renal biopsy).

The clinical data were thoroughly analyzed in 65 of the 81 patients with ANCA-associated glomerulonephritis. Forty-five of these patients had evidence of systemic vasculitis, including patients with the diagnostic criteria for Wegener's granulomatosis and polyarteritis nodosa. For 17 patients with ANCA-associated glomerulonephritis who had evidence of systemic disease, extrarenal tissues were available for examination by light, immunofluorescence, or electron microscopy, or all three, including 15 lung specimens, seven skin biopsies, eight nasal mucosa biopsies, and one autopsy.

For ANCA analysis, serum samples were obtained from over 200 patients who underwent renal biopsy evaluation at the University of North Carolina. In addition, 58 serum samples from IgA nephropathy patients were supplied by Dr. H. K. Yapp, National University of Singapore and Dr. S. C. Jordan, Cedars-Sinai Medical Center; 15 serum samples from patients with pathologically and serologically documented anti-GBM glomerulonephritis were supplied by Dr. A. J. Fish, University of Minnesota. Specimens from 83 blood bank donors served as normal controls.

ANCA Assays

ANCA were assayed in sera by both indirect immunofluorescence microscopy (IIFM) and enzyme immunoassay (EIA) using previously published methods.[10] Immunofluorescence microscopy preparations were determined to

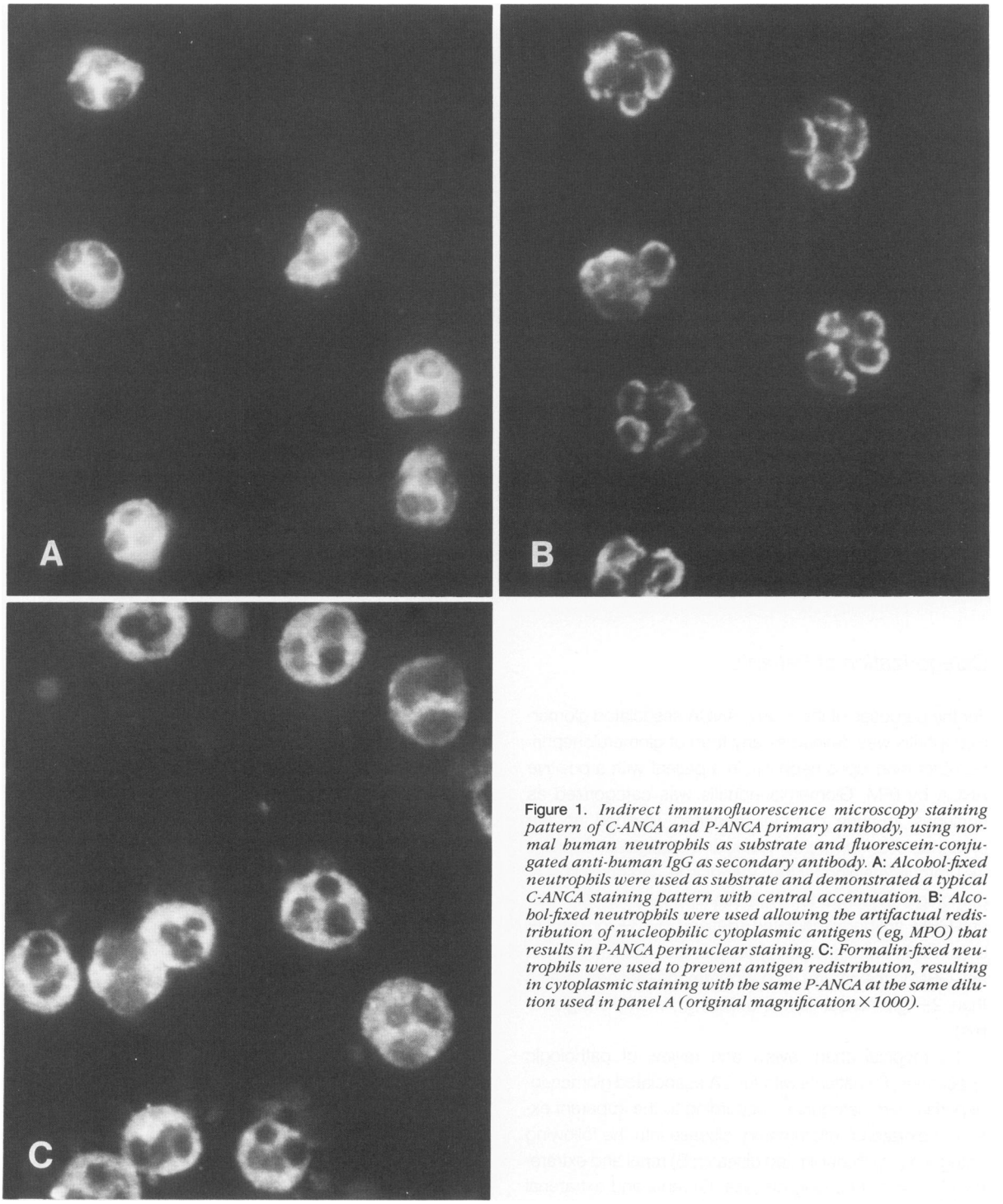

Figure 1. *Indirect immunofluorescence microscopy staining pattern of C-ANCA and P-ANCA primary antibody, using normal human neutrophils as substrate and fluorescein-conjugated anti-human IgG as secondary antibody.* **A**: *Alcohol-fixed neutrophils were used as substrate and demonstrated a typical C-ANCA staining pattern with central accentuation.* **B**: *Alcohol-fixed neutrophils were used allowing the artifactual redistribution of nucleophilic cytoplasmic antigens (eg, MPO) that results in P-ANCA perinuclear staining.* **C**: *Formalin-fixed neutrophils were used to prevent antigen redistribution, resulting in cytoplasmic staining with the same P-ANCA at the same dilution used in panel A (original magnification × 1000).*

be positive or negative; when positive, they were noted to have either a cytoplasmic (C-ANCA) or perinuclear (P-ANCA) (Figure 1A, B) pattern. These patterns distinguish between the two major types of ANCA. The artifactual perinuclear distribution of staining seen with P-ANCA is caused by the solubilization and binding of a nucleophilic cytoplasmic constituent, usually myeloperoxidase (MPO), to neutrophil nuclei during preparation of the alcohol-fixed substrate.[15] ANCA determinations were made without knowledge of the renal biopsy findings or clinical presentations of the patients.

A screening EIA[10] using an antigen substrate enriched for neutrophil cytoplasmic granule constituents that detects all ANCA was performed on all sera. A specific EIA

using myeloperoxidase (MPO)[10] was performed on 117 sera from 45 patients with P-ANCA-associated glomerulonephritis, 29 patients with C-ANCA glomerulonephritis, 16 patients with lupus nephritis, and 27 patients with no ANCA by IIFM. EIA values were expressed as the percentage of a standard positive control sample reaction. A standard negative control was evaluated in each assay, and the mean value from 33 assays was 19.1, with a standard deviation of 8.4. Triplicate EIA analysis of the preliminary ANCA International Serum Standard (obtained from Dr. Niels Rasmussen, Statens Seruminstitut, Copenhagen, Denmark) yielded a mean of 99.9 with a standard deviation of 17.2. Eighty-three normal blood bank samples were evaluated having a mean value of 23.2, with a standard deviation of 9.5. Patient results were considered positive when they were greater than two standard deviations above this mean value for normal blood bank donor samples, ie, greater than 42.2.

Serum samples from ANCA-associated glomerulonephritis patients were also evaluated by IIFM for antinuclear antibodies and anti-DNA antibodies using HEp-2 cells for the former and Crithedia luciliae for the latter (Behring Diagnostics, La Jolla, CA).

Categorization of Patients

For the purposes of this study, ANCA-associated glomerulonephritis was defined as any form of glomerulonephritis, other than lupus nephritis, in a patient with a positive ANCA by IIFM. Glomerulonephritis was categorized as nonlupus immune complex-mediated (defined as the presence of 2+ or greater granular mesangial or capillary wall immunostaining for IgG, IgA, or IgM, and no clinical or pathologic evidence for systemic lupus erythematosus), anti-GBM antibody-mediated (defined by 3+ or greater linear GBM immunostaining for IgG and confirmation of anti-GBM in the serum), or pauci-immune (defined as less than 2+ glomerular immunostaining for immunoglobulins).

By medical chart review and review of pathologic specimens, 65 patients with ANCA-associated glomerulonephritis were categorized according to the apparent extent of extrarenal inflammatory disease into the following categories: A) Renal-limited disease; B) renal and extrarenal disease but no lung disease; C) renal and extrarenal disease with lung but no sinus disease; D) renal and extrarenal disease with both lung and sinus disease.

Statistical Analysis

One-way analysis of variance was used to detect any differences among groups of patients with respect to the demographic, pathologic, and serologic parameters under study. When significant differences were detected among groups, Fisher's test for least significant differences was carried out to compare the groups.

Results

Association of ANCA with Pauci-Immune Necrotizing Glomerulonephritis and Systemic Necrotizing Vasculitis

As shown in Table 1 and Figure 2, ANCA were strongly associated with pauci-immune necrotizing and crescent-forming glomerulonephritis, but not with nonlupus immune complex-mediated or anti-GBM antibody-mediated glomerulonephritis. Both C-ANCA and P-ANCA IIFM staining patterns were identified in patients with pauci-immune glomerulonephritis. In 30 patients, 122 replicate positive sera were analyzed over time with no change in ANCA pattern in a given patient ever observed. At some time during follow-up, serum samples from 22 patients became negative for ANCA.

As shown in Figure 3, the P-ANCA pattern correlated with anti-MPO specificity, whereas the C-ANCA pattern did not. P-ANCA sera gave a mean MPO EIA value of 72.1 $\pm$ 22.2, which was significantly greater ($P < 0.01$) than the mean for C-ANCA sera (36.5 $\pm$ 12.0) and ANCA-negative sera (25.4 $\pm$ 11.1). The latter two means were not significantly different. Eighty-nine percent (40 of 45) of P-ANCA sera had an MPO EIA value greater than the negative control mean plus two standard deviations, compared with only 14% (four of 29) of C-ANCA sera.

For inclusion in the pauci-immune glomerulonephritis category, patients had to have either glomerular crescents or necrosis. Using these criteria, all but two of the 76 patients in the pauci-immune category had crescent formation and all but three had glomerular necrosis. The percentage of glomeruli with crescents ranged from 0% to 100%, with a mean of 43.8%. Of the 76 patients, 32 had 50% or more of glomeruli with crescents.

Of the 55 patients with nonlupus immune complex-mediated glomerulonephritis, 16 had crescent formation involving from 5% to 95% of glomeruli, including six patients with 50% or more glomerular crescents. Included in the immune complex-mediated glomerulonephritis category were 17 patients with IgA nephropathy and 22 patients with membranous glomerulopathy. The three patients in the immune complex category who had positive ANCA values by EIA were also positive by IIFM and only marginally met our working criteria for immune complex rather than pauci-immune glomerulonephritis. One of these patients had 2+ IgA glomerular immunostaining with small numbers of mesangial dense deposits and had acute sinusitis and pulmonary capillaritis with hemoptysis. The second had only 2+ IgG irregular glomerular immuno-

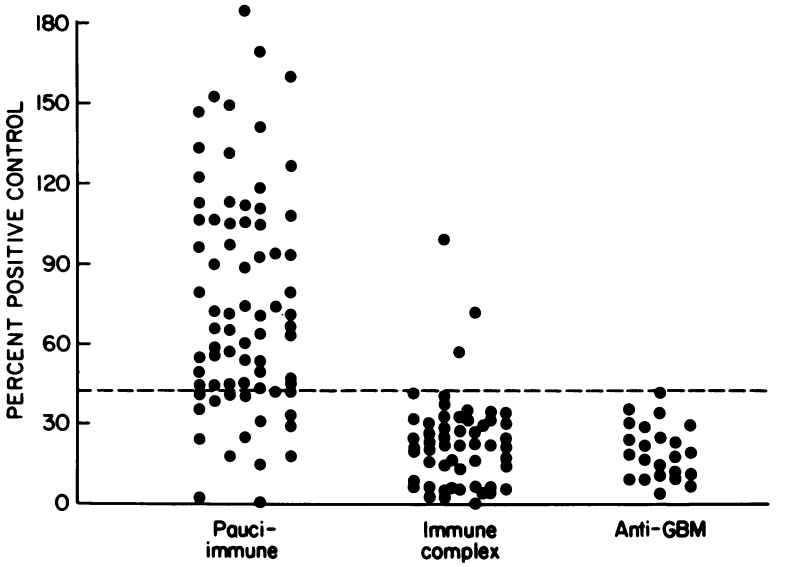

Figure 2. *Screening EIA ANCA values from 76 patients with pauci-immune glomerulonephritis with associated crescents or necrosis, 55 patients with nonlupus immune complex - mediated glomerulonephritis, and 24 patients with anti-GBM antibody-mediated glomerulonephritis. ANCA values are expressed as the percentage reactivity relative to a standard positive control. The dashed line represents the mean plus 2 SD value obtained for 83 normal blood bank donor specimens.*

staining with no electron dense deposits ultrastructurally. The third had necrotizing glomerulonephritis with 95% crescents, 2+ glomerular IgG, and scattered glomerular dense deposits.

An additional 58 sera from IgA nephropathy patients (provided by Dr. H. K. Yapp and Dr. S. C. Jordan) were analyzed by EIA for ANCA. These sera had a mean EIA value of 14.3 ± 10.8, with only one sample giving a positive value (ie, greater than 42.2). Nine positive sera run concurrently with the IgA nephropathy sera had a mean of 95.6 ± 23.3, and ten negative sera had a mean of 13.9 ± 10.2.

The anti-GBM glomerulonephritis group was comprised of nine patients diagnosed at the University of North Carolina and 15 patients diagnosed at the University of Minnesota. The diagnosis was confirmed in all patients both by renal biopsy and by serologic identification of anti-GBM antibodies.

Serum samples from 18 patients with biopsy-proven lupus nephritis were also analyzed. By IIFM, none had a C-ANCA pattern, but ten (56%) had nuclear staining that could not be readily distinguished from P-ANCA staining on alcohol-fixed cells. By the screening EIA, the lupus sera had a mean value of 76.0, and 14 (78%) were positive (ie, greater than 42.2). However, 16 sera from patients with biopsy-proven lupus nephritis gave a mean MPO EIA value of 38.4, which was not significantly different from the negative control mean of 25.4 and was significantly lower than the P-ANCA-associated pauci-immune glomerulonephritis mean of 72.1 ($P < 0.01$). Only two of 16 lupus patients had MPO EIA values in the positive range (ie, >control mean + 2 SD). Both positive lupus patients had over 50% crescent formation and segmental glomerular necrosis. One patient had severe hemoptysis with massive pulmonary consolidation radiographically.

Sera from 72 randomly chosen patients who underwent renal biopsy for diseases other than those already discussed were also analyzed by IIFM and EIA for ANCA. Of these, only four (6%) were positive by IIFM. The 72 sera had a mean EIA value of 23.1, and only eight

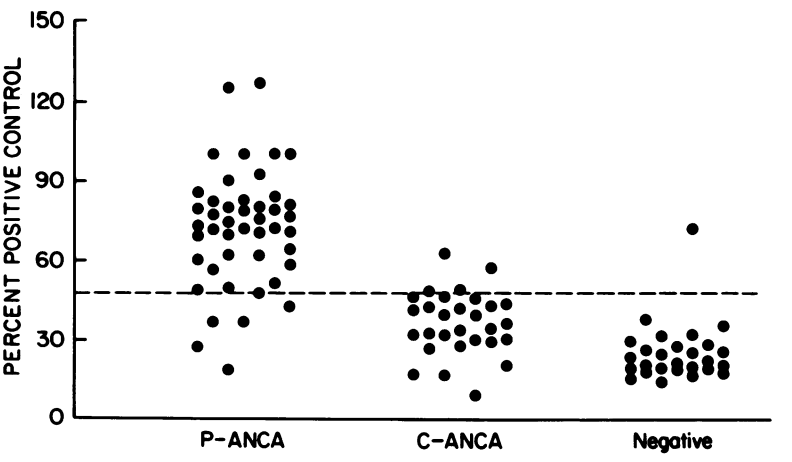

Figure 3. *EIA using purified MPO as antigen to assess the reactivity of P-ANCA sera from 45 patients, C-ANCA sera from 29 patients, and ANCA-negative sera for 27 patients. The dashed line represents the ANCA-negative sera mean plus 2 SD. Note the reactivity of P-ANCA but not C-ANCA with MPO.*

Table 2. *Pathologic Findings in Patients with ANCA-Associated Glomerulonephritis*

	All	C-ANCA	P-ANCA	A	B	C	D
Any crescents	75/81	32/34	43/47	17/20	12/12	20/21	10/12
Greater than 50% crescents (%)	30/81	11/34	19/47	9/20	4/12	7/21	1/12
Glomerular necrosis	76/81	32/34	44/47	17/20	11/12	21/12	12/12
Arteritis in the renal biopsy	10/81	4/34	6/47	0/20	3/12	0/21	3/12
Greater than 1+ immunoglobulin by immunofluorescence	10/71	3/31	7/40	4/18	0/11	1/18	1/11
Dense deposits by electron microscopy	11/69	5/31	6/38	3/16	0/11	2/17	1/11

A, no extrarenal disease; B, extrarenal but no lung disease; C, extrarenal with lung but no sinus disease; D, extrarenal with lung and sinus disease.

(11%) were considered positive (ie, had values greater than 42.2).

None of the 83 blood bank specimens gave a positive ANCA reaction by IIFM. These samples had a mean EIA of 23.2 with a standard deviation of 9.5. In separate EIA procedures, 33 determinations on the EIA standard negative control specimen yielded a mean value of 19.1 with a standard deviation of 8.4.

Frequency of ANCA-Associated Glomerulonephritis

From our data we cannot directly determine the frequency of ANCA-associated glomerulonephritis relative to other forms of glomerulonephritis; however, we can infer this from the frequency of ANCA in patients with the three major categories of glomerulonephritis (ie, immune complex-mediated, anti-GBM antibody-mediated, and pauci-immune) and from the relative frequency of the three major categories of glomerulonephritis among all patients with glomerulonephritis.

Of 1500 consecutive nontransplant renal biopsies, 15% had some degree of crescent formation. Of glomerulonephritis with any degree of crescent formation, 51% were pauci-immune, 44% immune complex-mediated, and only 5% anti-GBM antibody mediated. The frequency of pauci-immune glomerulonephritis was even greater in patients with greater than 50% crescent formation (50%), glomerular necrosis (60%), or arteritis in the renal biopsy specimen (81%). Because approximately 80% of patients with pauci-immune glomerulonephritis with crescents have ANCA, it can be inferred that ANCA-associated glomerulonephritis is the most common form of crescent-forming glomerulonephritis.

Characteristics of ANCA-Associated Glomerulonephritis

The pathologic findings in 81 patients with glomerulonephritis and positive IIFM serology for ANCA were evaluated (Table 2). By definition, all 81 patients had ANCA by IIFM; 34 had C-ANCA, and 47 had P-ANCA. Their mean screening EIA level was 83.7 ± 36.8 with 89.0% having a value greater than the control mean plus 2 SD.

The 81 patients ranged in age from 2 to 86 years, with a mean of 57 years (±17.5) and a median of 60 years. The male-to-female ratio was 43 to 38. At the time of biopsy, the patients had a mean creatinine of 5.8 ± 4.6 mg/dl (range, 0.7 to 20.1). Of 79 patients tested for antinuclear antibodies using HEp-2 cells, 14 (18%) were positive at a serum dilution of 1:20 and produced a variety of patterns. Of 79 patients tested for anti-DNA antibodies using Crithedia luciliae, none were positive at a serum dilution of 1:20.

Based on the immunohistologic criteria, none of the 81 had anti-GBM antibody-mediated glomerulonephritis and only four met the criteria set for immune complex-mediated glomerulonephritis. All of the latter had no more than 2+ immunostaining for immunoglobulin and had only relatively small scattered electron dense deposits ultrastructurally.

The most frequent histologic appearance was focal segmental glomerular fibrinoid necrosis with crescent formation and only mild hypercellularity (Figures 4 and 5). Neutrophils in general were not conspicuous but were frequently present at sites of necrosis. In a few cases, a granulomatous response was noted adjacent to glomeruli that had necrosis and disruption of Bowman's capsule (Figure 5).

By immunofluorescence microscopy, only 14% of 71 patients with ANCA-associated glomerulonephritis had greater than 1+ immunostaining for any immunoglobulin and these cases had only 2+ staining. When present, immunoglobulin staining was usually predominantly within the mesangium. Irregular immunostaining for fibrin was present in necrotic segments and within crescents. Irregular immunostaining for C3, C1q, and IgM was present at sites of glomerular sclerosis.

By electron microscopy only 16% of 69 patients with ANCA-associated glomerulonephritis had any glomerular electron dense deposits; these were relatively small, scattered deposits, most often confined to the mesangium. No endothelial tubuloreticular inclusions were identified. In necrotic segments, disruption of glomerular basement

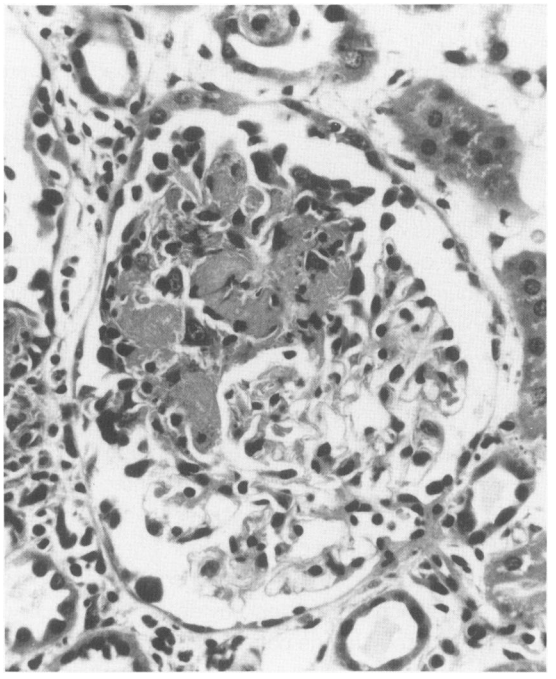

Figure 4. *ANCA-associated glomerulonephritis with characteristic segmental fibrinoid necrosis (H&E, original magnification ×375).*

membranes was noted. This was often associated with capillary thrombosis and extension of fibrin tactoids into adjacent crescents. Occasional glomerular capillaries, especially in necrotic segments, contained marginated neutrophils and mononuclear phagocytes.

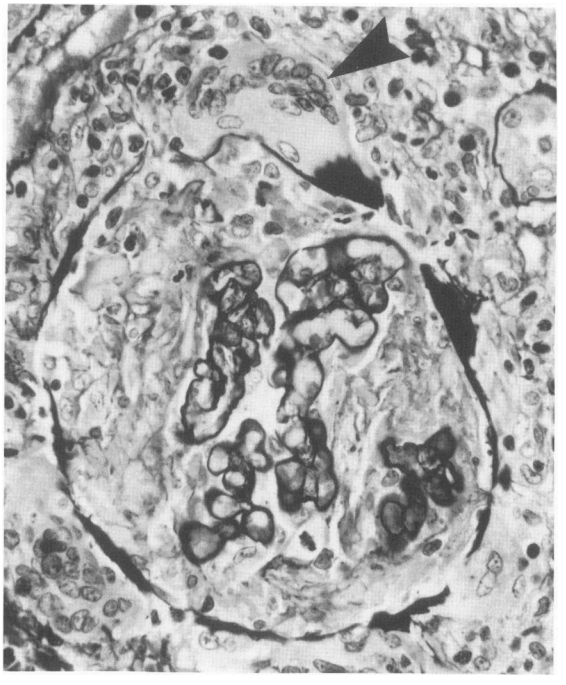

Figure 5. *ANCA-associated glomerulonephritis with crescent formation, focal disruption of Bowman's capsule, and a periglomerular granuloma with multinucleated giant cells (arrow) (Silver methenamine, original magnification ×460).*

Table 3. *Correlation of Extrarenal Disease Distribution with ANCA**

	All	A	B	C	D
C-ANCA-GN	34 (42%)	4 (20%)	7 (58%)	8 (38%)	8 (67%)
P-ANCA-GN	47 (58%)	16 (80%)	5 (42%)	13 (62%)	4 (33%)
Total	81 (100%)	20 (100%)	12 (100%)	21 (100%)	12 (100%)

* Determined in 81 patients with ANCA-associated GN, 65 of whom were categorized on the basis of clinical data into categories A (no extrarenal disease), B (extrarenal but no lung disease), C (extrarenal and lung but no sinus disease), and D (extrarenal with lung and sinus disease).

Table 2 indicates that there were no substantial differences in the pathologic glomerular changes between patients with C-ANCA *versus* P-ANCA, or among patients with different degrees of extrarenal vascular inflammation. Periglomerular granulomas were observed more often in patients with C-ANCA, although most patients with C-ANCA did not have periglomerular granulomas. There was a small tendency for P-ANCA patients to have slightly more glomerular immune deposition than C-ANCA patients; patients with renal-limited disease, likewise, had slightly more glomerular immune deposition than patients with systemic disease. In all groups, however, by far the preponderant characteristic was the paucity of immune deposits.

Distribution of Systemic Disease in Patients with ANCA-Associated Glomerulonephritis

Table 3 summarizes the distribution of extrarenal disease in patients with ANCA-associated glomerulonephritis, and correlates this with the ANCA type. Thorough clinical, laboratory, and pathologic data were available on 65 patients that permitted them to be categorized into groups A, B, C, or D based on the distribution of disease. Because of our selection method, all patients had glomerulonephritis. Thus, the frequency of the various distributions of disease in ANCA-positive patients only pertains to patients with ANCA-associated glomerulonephritis.

The data in Table 3 indicate that patients with ANCA-associated glomerulonephritis with no evidence for systemic disease, a category that would include most patients with so-called idiopathic or primary crescentic glomerulonephritis, most often had P-ANCA rather than C-ANCA. Nevertheless, some patients with C-ANCA had glomerulonephritis with absolutely no evidence of extrarenal disease. Patients with lung and sinus involvement had the highest frequency of C-ANCA relative to P-ANCA, but even in this group some patients had P-ANCA. As will be discussed later, pathologically documented ANCA-asso-

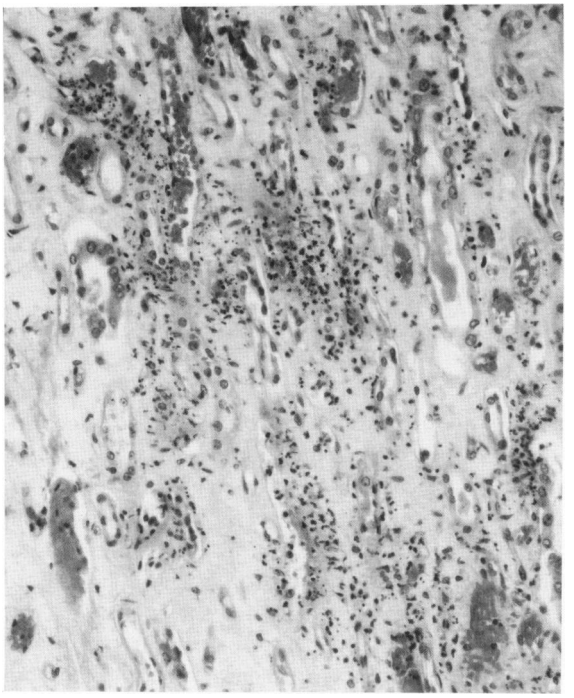

Figure 6. *ANCA-associated renal medullary leukocytoclastic capillaritis (H&E, original magnification ×200).*

ciated granulomatous respiratory tract inflammation may be strongly correlated with C-ANCA, although patients with alveolar capillaritis can have C-ANCA or P-ANCA. Necrotizing arteritis in the renal biopsy was not confined to any one of the clinical or serologic categories.

Pathologic Characteristics of ANCA-Associated Systemic Vasculitis

In patients with ANCA-associated disease, varying degrees of necrotizing inflammation of capillaries, venules, arterioles, and arteries were observed in the kidneys, lungs, skin, nasal mucosa, and other organs. A ubiquitous feature was the presence of vascular fibrinoid necrosis, usually accompanied by leukocytic infiltration with varying degrees of leukocytoclasia. At some sites either neutrophils or mononuclear leukocytes were predominant, whereas at others there were admixtures of both. One patient, who did not have asthma, had conspicuous eosinophils within the inflammatory infiltrates. Examination by immunofluorescence microscopy of skin and lung tissue with vasculitis demonstrated no immune complex deposits. Examination by electron microscopy of lung tissue with alveolar capillaritis revealed no vascular electron dense deposits.

Three kidney biopsy specimens were found to have a medullary peritubular necrotizing leukocytoclastic capillaritis (Figure 6). The frequency of this lesion's occurrence cannot be determined because of its focal nature and the lack of medullary tissue in many of the biopsies.

Of seven skin biopsies examined, five had a severe necrotizing leukocytoclastic angiitis, one had a panniculitis with necrotizing arteritis, and one had perivascular scarring and chronic inflammation.

Table 4 summarizes the respiratory tract lesions identified in patients with ANCA-associated glomerulonephritis. The most frequent lung lesion was a striking alveolar capillaritis (Figure 7) characterized by marked margination of neutrophils along alveolar capillaries and focal necrosis of alveoli with spillage of neutrophils into alveolar spaces. This lesion was associated with extensive alveolar hemorrhage, and the patients characteristically had hemoptysis, dyspnea, and pulmonary infiltrates radiographically.

Pulmonary granulomas were documented pathologically in four patients. In three of these, the lesions were granulomas characteristic of Wegener's granulomatosis with central irregular necrosis, degenerating neutrophils, and marginal mononuclear leukocytes, including multinucleated giant cells. In one patient, however, the granulomas were more discrete and were found to contain mycobacteria both histochemically and by culture. The first three patients all had C-ANCA and the latter patient had P-ANCA. Therefore, all three patients with Wegener's-type granulomas had C-ANCA. However, not all patients with C-ANCA had granulomas at the sites of vasculitis and inflammation. Seven patients with C-ANCA had alveolar capillaritis without granulomas, including one C-ANCA patient who at autopsy was found to have a pauci-immune focal necrotizing and crescent-forming glomerulonephritis and severe hemorrhagic necrotizing alveolar capillaritis (with no immune deposits by immunofluorescence microscopy) but no granulomatous inflammation or arteritis.

Alveolar interstitial fibrosis was present alone or was associated with granulomas or capillaritis in most patients. Some patients with alveolar capillaritis showed an apparent transition in some areas between the acute injury and a pattern of sclerosing diffuse alveolar damage, usually accompanied by hemosiderin-laden macrophages. In one patient, an initial lung biopsy showed acute alveolar capillaritis with hemorrhage, whereas a subsequent biopsy showed only diffuse interstitial fibrosis.

Eight nasal biopsies were evaluated. Patients with either P-ANCA or C-ANCA frequently had a lesion manifesting as a necrotizing acute inflammation, often with conspicuous margination of neutrophils along the microvasculature. Two nasal biopsies from patients with C-ANCA had granulomas and one had a necrotizing arteritis.

Discussion

Our data show that both C-ANCA and P-ANCA are associated with necrotizing vasculitis affecting vessels ranging

Table 4. *Pathologic Findings in 15 Lung Specimens**

Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ANCA type	C	C	C	P	C	C	P	C	C	C	C	C	P	C	C
Lung tissue															
Specimen type	O	T	O	O	O	A	O	O	O	O	O	T	O	O	R
Granulomas	+	+	+	+†	−	−	−	−	−	−	−	−	−	−	−
Capillaritis	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−
Hemorrhage	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−
Fibrosis	+	+	+	+	−	−	−	−	+	+	+	+	+	+	+
Nasal tissue															
Granulomas	+	N	−	N	N	N	−	−	N	N	N	N	N	N	−
Necrotizing acute inflammation	+	N	−	N	N	N	+	+	N	N	N	N	N	N	+

* Five of the specimens had accompanying nasal mucosa. The specimens were from patients with ANCA-associated glomerulonephritis and lung disease.

† Most likely tuberculous granulomas.

O, open biopsy; T, transbronchial biopsy; A, autopsy specimen; R, resected lobe; N, not available.

in size from capillaries to muscular arteries in many different tissues. Based on clinicopathologic criteria, some of our 81 patients with ANCA-associated glomerulonephritis would be diagnosed as having idiopathic (primary) crescentic glomerulonephritis, polyarteritis nodosa, or Wegener's granulomatosis. However, most fall within a continuum of clinicopathologic features extending from renal-limited disease to extensive extrarenal disease. By definition, all of the patients in our study had nephritis, but other researchers have reported patients with ANCA-associated disease and no apparent renal involvement.[12] We propose that all patients who have ANCA may share a common pathogenetic mechanism that causes different distributions of vascular injury in different patients and in the same patients at different times.

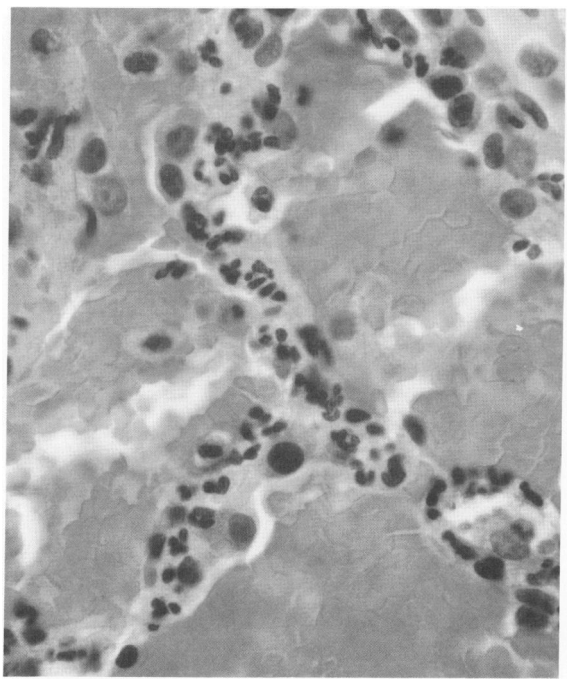

Figure 7. *ANCA-associated pulmonary alveolar capillaritis. Note the striking influx of neutrophils and the massive hemorrhage into the alveolar spaces (H&E, original magnification ×750).*

There has been some debate about the diagnostic specificity of C-ANCA for Wegener's granulomatosis.[12] There is no doubt that C-ANCA is a sensitive marker for active Wegener's granulomatosus when this disease is defined by criteria that include the pathologic identification of granulomas in the respiratory tract. However, our data and that of others indicate that not all patients with C-ANCA can be identified as having Wegener's granulomatosis by any previously recognized criteria. The proportion of C-ANCA patients without feature's of Wegener's granulomatosis who will eventually develop such features is unknown.

We have unpublished data suggesting that ANCA are not only associated with vasculitis but in fact are involved in the pathogenesis of necrotizing vasculitis. *In vitro,* IgG isolated from P-ANCA- or C-ANCA-positive sera cause a respiratory burst and degranulation of viable normal human neutrophils. This event is facilitated by first priming the neutrophils. Priming is known to cause the release of small amounts of primary granule constituents at the cell surface that can then interact with ANCA to cause total activation with release of toxic oxygen radicals and lytic enzymes. We postulate that, in patients with ANCA, a triggering event, such as a viral infection, primes neutrophils in the circulation allowing for ANCA-induced neutrophil activation with resultant vascular injury. The site of vascular injury may be influenced by local concentrations of cytokines or by the physiology of leukocyte trafficking through a given vessel. For example, the propensity for patients with ANCA to develop upper and lower respiratory tract vasculitis after viral respiratory tract infections could be a result of the availability of cytokine-primed neutrophils and mononuclear phagocytes at the site of viral infection.

In conclusion, ANCA are associated with pauci-immune necrotizing glomerulonephritis and systemic vascu-

litis. ANCA should be a useful diagnostic serologic marker for these diseases, and may be helpful in monitoring the course of the disease and response to treatment. ANCA of different specificities are associated with different distributions of vasculitis. It is possible that ANCA are directly involved in a novel pathogenetic mechanism that is responsible for causing common forms of crescentic glomerulonephritis and necrotizing systemic vasculitis.

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