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Crescentic Glomerulonephritis
: A Clinicopathologic Analysis of 17 Cases with Emphasis on Glomerular and Interstitial Neutrophil Infiltration

In order to determine the extent to which specific forms of glomerulonephritis (GN) contribute to the pool of crescentic GN, renal tissues from 17 crescentic GN patients were examined with special attention to glomerular and interstitial neutrophil infiltration. Renal tissues from five normal kidneys served as normal controls. Renal biopsy tissues from five patients with postinfectious GN in which crescent formation was not observed were also examined as disease controls. The patients were put into both three groups according to immunofluorescence findings and two groups according to the active or inactive phase of the crescents: group 1 with anti-glomerular basement membrane crescentic GN, one case; group 2 with immune complex crescentic GN, ten cases; and group 3 with pauci-immune crescentic GN, six cases. Four of the nine individuals tested were positive for anti-neutrophil cytoplasmic antibody (44.4%). Glomerular and interstitial neutrophil infiltrations were prominent in both the active and inactive phase groups, compared to normal controls ($p<.05$). Glomerular neutrophil infiltration was significantly prominent in the active phase group, compared to the inactive phase group ($p<.001$). In both the active and inactive phase groups, interstitial neutrophil infiltration was prominent, compared to disease control groups ($p<.05$). These results support the concept of the participation of periglomerular leukocytes in the renal tissue damage of crescentic GN, although the role of neutrophils was not examined.

Key Words: *Glomerulonephritis, crescentic; Fluorescent antibody technique; Antibodies, anti-neutrophil cytoplasmic; Neutrophils*

Kwang-Sun Suh, Beum-Kyeong Kim, Kyeong-Hee Kim

Department of Pathology, Chungnam National University School of Medicine, Taejon, Korea

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Address for correspondence
Kwang-Sun Suh, M.D.
Department of Pathology, Chungnam National University School of Medicine, 6, Munwha-dong, Jung-gu, Taejon 301-131, Korea
Tel: +82.42-220-7199, Fax: +82.42-220-7189
E-mail: kssuh@hanbat.chungnam.ac.kr

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INTRODUCTION

It is widely known that almost any form of glomerular disease may be complicated by the formation of crescents. Crescentic glomerulonephritis (GN) is a heterogeneous collection of clinicopathologic entities which are unified by the presence of epithelial proliferation in Bowman's space (1). This occurs in anti-glomerular basement membrane (GBM) antibody disease and specific forms of GN, such as membranous, membranoproliferative, IgA, and postinfectious GN. Crescents are also significant in mixed essential cryoglobulinemia, systemic lupus erythematosus (SLE), Henoch-Schoenlein purpura, polyarteritis nodosa, and Wegener's syndrome (2).

The pathogenesis of this entity has yet to be fully elucidated. The escape of fibrin into Bowman's space has been accepted as the most likely mechanism in crescent formation. The high frequency of a positive test for anti-neutrophil cytoplasm antibodies (ANCA), which are usually detected in vasculitic diseases (3), and the similarities of renal histologic manifestations suggest that common pathological mechanisms are involved in both crescentic GN and systemic vasculitis. Recently, it has been demonstrated that renal infiltration of neutrophils was upregulated in systemic vasculitis by vascular endothelial leukocyte adhesion molecule-1 expression, which suggested neutrophil-dependent renal tissue injury in this disease (4). It has been suggested that neutrophil elastase plays a significant role in renal tissue damage, especially in the formation of glomerular necrotizing and crescentic lesions, and in periglomerular interstitial lesions of crescentic GN (5).

In order to determine the extent to which specific forms of GN contribute to the pool of crescentic GN,

samples from our renal biopsy service were analyzed. In addition, renal tissues from crescentic GN patients were analyzed with special attention to glomerular and interstitial neutrophil infiltration.

MATERIALS AND METHODS

Patients

The diagnosis of crescentic GN was based on histologic findings of more than 50% glomerular involvement by crescents, along with typical clinical manifestations and laboratory data. The mean number of glomeruli per case was 15.3 (range 6-30). Of the renal needle biopsy specimens examined by light, immunofluorescence, and electron microscopy at the Department of Pathology, Chungnam National University Hospital, between January 1990 and December 1997, 17 cases satisfied the above criteria. Serum ANCAs were analyzed in nine cases by indirect immunofluorescence microscopy.

Three groups of crescentic GN

The studied 17 cases were divided into three groups according to immunofluoroscence findings (2): group 1 with linear fluorescence for IgG with or without C3 (anti-GBM crescentic GN); group 2 with granular fluorescence for immunoglobulins and complement along the glomerular capillary walls and/or in the mesangium (immune complex crescentic GN); and group 3 with negative or only localized positive fluorescence for immunoglobulins and/or complement (pauci-immune crescentic GN).

Active or inactive phase crescents

The studied 17 patients were divided into two groups according to the active or inactive phase of the crescents (1). Active phase crescents are characterized by predominantly cellular crescents, intermingled with fibrin and polymorphs, or necrotizing lesions. Inactive crescents are generally organized, fibrous, or fibrocellular and lack fibrin deposition. The two groups were designated as crescentic A (with active crescents; n=9) and cresentic I (with inactive crescents; n=8).

The degree of neutrophil infiltration

Renal tissues from five normal kidneys that had been surgically removed for localized tumors served as normal controls. Renal needle biopsy tissues from five patients with postinfectious GN in which crescent formation was not observed were also examined as disease controls. The degree of glomerular infiltration was semiquantitatively assessed under light microscopic examination in crescentic glomeruli of each crescentic GN group and in glomeruli without crescents of both normal and disease controls (5). Glomerular neutrophil infiltration was scored from 1 to 4 according to the mean number of glomerular infiltrating cells in each glomerular cross section: score 1, 0 to <2; score 2, 2 to <4; and score 3, 4 to <6; and score 4, ≥6/glomerular cross section. The degree of periglomerular interstitial neutrophil infiltration was semiquantitatively assessed in each crescentic GN group and in both normal and disease controls (5). Interstitial neutrophil infiltration was also scored from 1 to 4: score 1, absent or rare; score 2, focal sparse infiltration; score 3, focal dense infiltration; and score 4, diffuse dense infiltration (Table 1). Mean grade values of glomerular and interstitial neutrophil aggregation were calculated for each group.

Table 1. Scoring system of glomerular and interstitial neutrophil infiltration

Score	Glomerular cross section	Interstitial infiltration
1	0 to <2	absent or rare
2	2 to <4	focal, sparse
3	4 to <6	focal, dense
4	≥6	diffuse, dense

Statistical analysis

Data are expressed as mean±SD. The degree of glomerular and interstitial neutrophil infiltrations among groups was analyzed using the Student's t-test. Differences were considered significant if p value is less than 0.05.

RESULTS

Clinicopathologic findings and classification of crescentic GN

All patients had renal impairment at presentation with a mean serum creatinine levels of 5.3±5.2 mg/dL (range 1.9-20.5 mg/dL). The mean patient age was 47.0±17.6 yr (range; 21 to 65) and male to female ratio was 1.1: 1. The mean percentage of glomeruli involving crescents was 70.5±17.6 (range; 50% to 100%).

These 17 cases were divided into three groups according to the immunofluoroscence picture: group 1 with anti-GBM crescentic GN, one case; group 2 with immune complex crescentic GN, ten cases; and group 3

Table 2. Three groups of crescentic glomerulonephritis

	Group 1	Group 2*	Group 3	Total
No. of cases	1	10	6	17
Sex (M:F)	1:0	2.3:1	1:5	1.1:1
Age (years)				
Range	57	21-60	27-65	21-65
Mean±SD	-	48.3±18.6	42.4±18.2	47.0±17.6
Creatinine (mg/dL)	4.8	4.2±3.8	8.9±6.7	5.8±5.1
Crescent (%)	71.4	76.7±18.8	58.1±9.0	70.5±17.6
ANCA (+)	n.d.†	-(5 cases)	4†	4

*includes specific identifiable forms of glomerulonephritis.
†n.d.: not done.
†2 cases: c-ANCA+, 2 cases; p-ANCA+

with pauci-immune crescentic GN, six cases (Table 2). Among the ten cases in group 2 the specific contributing conditions were identified as follows: three cases of post-infectious GN, one case of IgA nephropathy, one case of membranoproliferative GN, three cases of Henoch-Schoenlein purpura nephritis, and two cases of SLE with cryoglobulinemia (Table 3). Of the nine patients with crescentic GN who were tested with serum ANCA, two patients were positive for cytoplasmic-ANCA and two were positive for perinuclear-ANCA. These four ANCA-positive cases were all from group 3 (Table 2), showing segmental necrotizing and/or diffuse or segmental proliferative changes in the tufts accompanied by variable numbers of crescents. Necrotizing vasculitis was seen in one out of three Henoch-Schoenlein purpura nephritis cases. Granulomatous vasculitis was present in one post-transplant kidney which was c-ANCA positive. This case is considered as Wegener's granulomatosis.

Table 3. Crescentic glomerulonephritis with specific identifiable forms of glomerulonephritis

Specific forms of glomerulonephritis	Number of cases
Primary (idiopathic) crescentic GN	6
Anti-GBM antibody disease	1
Complicating various forms of glomerulonephritis	5
IgA nephropathy	1
Postinfectious	3
Membranoproliferative	1
Systemic disease	5
Henoch-Schoenlein purpura	3
Systemic lupus erythematosus	2
Total	17

Table 4. Degree of glomerular and interstitial neutrophil infiltration

Group	No. of cases	Glo. neutro.	Int. neutro.
Normal control	5	1	1
Disease control	5	3.8±0.4	1.2±0.4
Crescentic active	9	3.3±0.7*	3.8±0.7*†
Crescentic inactive	8	1.6±0.5*	2.8±0.7*†

Abbreviations: Glo. neutro., glomerular neutrophil infiltration; Int. neutro., interstitial neutrophil infiltration.
*$p<0.05$ v normal.
†$p<0.05$ v disease control.

Glomerular and interstitial neutrophil infiltration

The degree of glomerular and interstitial neutrophil infiltration in each patient group is summarized in Table 4. Glomerular and interstitial neutrophil infiltrations were prominent in both the active and inactive phase groups, compared to the normal controls ($p<0.05$) (Fig.

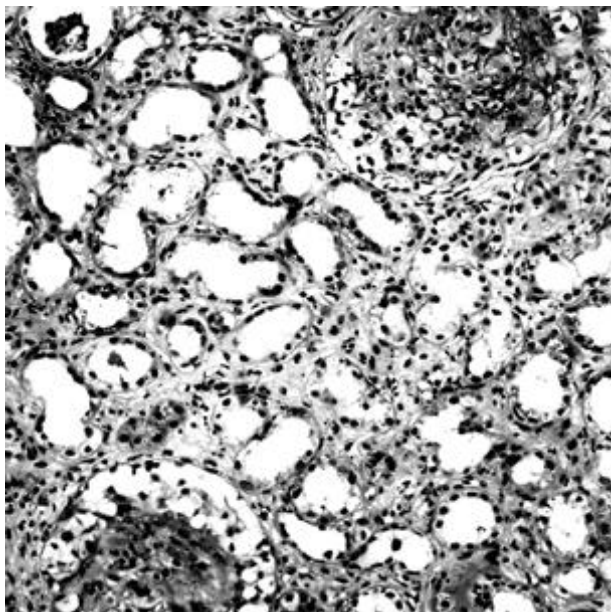

Fig. 1. Crescentic glomerulonephritis showing active cellular crescents. Glomerular and interstitial neutrophilic infiltrations are prominent (H&E, ×100).

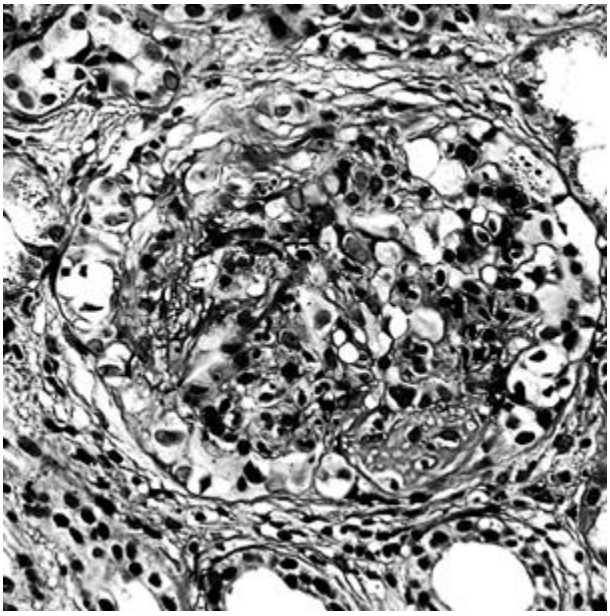

Fig. 2. Crescentic glomerulonephritis showing active cellular crescents. There is prominent neutrophilic infiltration in the glomerular tufts and cellular crescents (PAS, ×200).

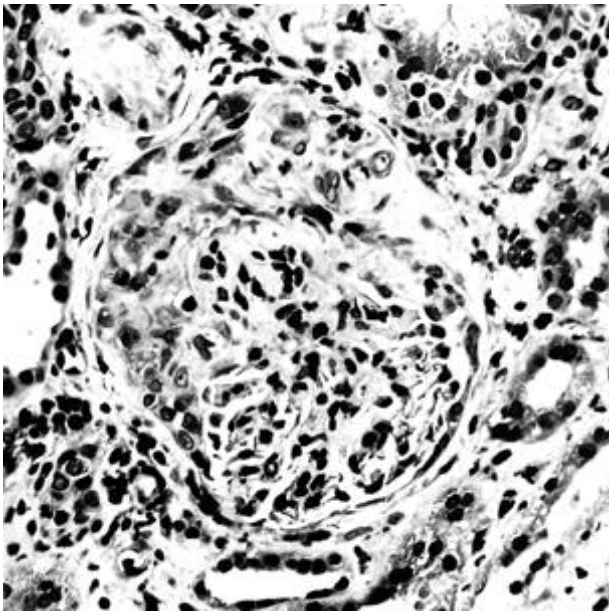

Fig. 3. Crescentic glomerulonephritis showing inactive fibrocellular crescents. A few neutrophils are seen in the fibrocellular crescent. There is a diffuse periglomerular infiltration of mononuclear cells and neutrophils (H&E, ×200).

1). Glomerular neutrophil infiltration was significantly prominent in the active phase group compared to the inactive phase group ($p<0.001$) (Fig. 2, 3). In both active and inactive phase groups, interstitial neutrophil infiltration was prominent, compared to the disease control groups ($p<0.05$).

Follow-up data

One patient who had crescentic GN due to IgA nephropathy died of the disease two months after examination. One patient with ANCA-related crescentic GN received a renal transplantation. The function of the graft remained good without relapse of the underlying disease. We described this case elsewhere (6).

DISCUSSION

The number of crescents required to justify diagnosis of crescentic GN varies from author to author. Some use 30 percent or more of glomeruli showing crescents (7, 8), while others use 50 percent or more (9-11). In this study, we included cases which had at least 50 percent of glomeruli containing crescents or more, and the majority of included cases had over 70 percent involved.

We classified the 17 cases of crescentic GN into three groups according to immunofluoroscence results: Group 1 with linear fluorescence for IgG, one case; Group 2 with granular fluorescence for immunoglobulins and complement, ten cases; and Group 3 with negative or only localized positive fluorescence, six cases. The immunofluorescence pattern of Group 1 was identical to the pattern which is accepted as indicating anti-GBM antibody disease (2). One patient from Group 1 in this study suffered from hemoptysis, but the serologic test for anti-GBM antibody was not performed. In Group 2, there were significant amounts of immunoglobulins and complement in ten cases. These ten cases were allocated to a specific form of immune complex GN. Group 3 did not contain any of the specific forms of GN. The possibility remains that some of the cases were examples of generalized vasculitis (2). One out of the six cases in Group 3 showed granulomatous vasculitis.

The presence of ANCA in the serum has been described and is widely prevalent in Group 3 (sometimes called the pauci-immune group) (2, 12, 13). In 1982, ANCA was first reported in eight patients with pauci-immune necrotizing GN (12). ANCA has been recognized as a useful tool in the diagnosis of vasculitis as Wegener's granulomatosis, microscopic polyangiitis, and "idiopathic pauci-immune" necrotizing crescentic GN. The presence of ANCA in four patients with systemic vasculitis, three of whom had necrotizing GN, has been reported (13). It has been confirmed that ANCA is a sensitive test for Wegener's granulomatosis and the titer of ANCA correlates with clinical disease activity (14, 15). ANCA was also found in patients with microscopic

polyarteritis nodosa, Churg-Strauss syndrome, and idiopathic crescentic GN (16, 17). ANCA was also detected in 9% of postinfectious GN patients (18). The presence of ANCA was significantly associated with a more severe glomerular disease, as assessed by serum creatinine and crescent formation. Of the nine patients with crescentic GN who were evaluated for the presence of ANCA in this study, two patients were positive for cytoplasmic-ANCA and two were positive for perinuclear-ANCA. These four ANCA-positive cases belonged to Group 3. One c-ANCA positive case was a renal transplant accompanied by granulomatous vasculitis. We considered this case as Wegener's granulomatosis.

Recently, it has been suggested that neutrophils are abundant in crecentic GN and that neutrophil elastase (NE) is involved in the destruction of glomerular tufts, and in the subsequent formation of necrotizing and crescentic lesions in crescentic GN (5). In this study, glomerular and interstitial neutrophil infiltration was prominent in both the active and the inactive phase groups, compared to the normal controls ($p<0.05$). Glomerular neutrophil infiltration was significantly prominent in the active phase group compared to the inactive phase group ($p<0.001$). NE is a highly cationic substance (19, 20) so it easily binds to and degrades the anionic GBM. No extracellular NE deposition is seen in acute postinfectious GN, which is seldom accompanied by glomerular crescents, in spite of dense NE-positive neutrophil infiltration (5). Extracelluar NE is usually accompanied by neutrophil aggregations but can also be observed in the periglomerular site, even with limited neutrophil infiltration (5). We found no significant difference in glomerular neutrophil infiltration between the crescent group and the disease control group. However, interstitial neutrophils were densely aggregated in both the active and inactive phase crescent groups, compared to the disease control group ($p<0.05$). The dense infiltration of interstitial neutrophils suggests the possibility of increased deposition of extracellular NE. The significance of interstitial neutrophil infiltration in the renal tissue injury of crescentic GN can also be explained as follows: In severe necrotizing GN, which occurs most often with anti-GBM GN and ANCA-GN, gaps in Bowmans capsule can allow leukocytes, especially macrophages, to enter Bowman's space from the interstitium. Perforation of Bowman's capsule may be caused by leukocytes within Bowman's space and by periglomerular inflammatory cells (21).

Neutrophils and NE are known not only to degrade tissues, but in the cascade of immuno-inflammatory processes, to play a role at sites of inflammation by modulating immune reactions of lymphocytes and macrophages (22, 23). Such mechanisms may be involved in the renal tissue damage of crescentic GN. It has been reported that intraglomerular neutrophil infiltration was significant in the acute phase of IgA nephropathy, which is often associated with macroscopic hematuria and crescent formation (24). Increased urinary excretion of neutral proteinase along with GBM fragments has been described in patients with a rapidly progressive GN (25). NE-alpha-1-proteinase inhibitor (α-1-PI) complex in plasma has been well documented to reflect the extracellularly released NE and is used as a marker of neutrophil activation and degranulation (26). Therefore, NE-α-1-PI complex in urine may reflect the extracellularly released NE in renal tissue in situ (5).

Recent studies in experimental proliferative GN indicate that apoptosis plays an essential role in the resolution of intra- and extraglomerular inflammation and in the elimination of glomerular cells within the scarring regions for progressive crescentic GN. The regulation of the apoptotic phenomenon during crescentic GN may be important in the progression of glomerular inflammation and development of pathologic glomerular sclerosis (27). In addition, several adhesion molecules, such as the intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and endothelial leukocyte adhesion molecule-1, are expressed on cells forming crescents and are modified during crescent evolution. These molecules are up-regulated on endothelial cells in relation to the severity of the inflammatory reponse and whatever the mechanism of the GN, the adhesion molecule expression is identical. It has been postulated that adhesion molecules play a role in crescentic GN (28).

In summary, prominent glomerular and interstitial infiltration of neutrophils has been shown in this study of crescentic GN. These results support the concept of the participation of periglomerular leukocytes in the renal tissue damage of crescentic GN, even though the immunohistochemical approach for NE staining was not performed.

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