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Original contribution

# Claudin 1 and nephrin label cellular crescents in diabetic glomerulosclerosis☆

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**Summary** Cellular crescents are typically inflammatory and associated with rapidly progressive glomerulonephritis. Their pathogenesis involves glomerular basement membrane rupture due to circulating or intrinsic factors. Crescents associated with diabetic glomerulosclerosis are rarely reported. Furthermore, the nature of cells forming crescents in diabetes is unknown. To investigate the nature of crescents in diabetes, we examined renal biopsies from diabetic patients with nodular glomerulosclerosis and crescents (n = 2), diabetes without crescents (n = 5), nondiabetic renal biopsies (n = 3), and crescentic glomerulonephritis with inflammatory crescents (n = 5). Electron microscopy and confocal immunofluorescence analysis with antibodies against nephrin (a podocyte marker) and claudin 1 (parietal epithelial cell marker) were performed. Diabetic glomeruli with crescents contained a mixture of crescentic cells expressing either claudin 1 (11 ± 1.4 cells/glomerulus) or nephrin (5.5 ± 3.0 cells/glomerulus). Rare crescentic cells coexpressed nephrin and claudin 1 (2.5 ± 1.6 cells/glomerulus). In contrast, inflammatory crescents were almost exclusively composed of claudin 1–positive cells (25 ± 5.3 cells/glomerulus). Cells coexpressing claudin 1 and nephrin were absent in inflammatory crescents and all cases without crescents. Electron microscopy showed podocyte bridge formation between the glomerular basement membrane and parietal basement membrane but no glomerular basement membrane rupture as in inflammatory crescents. Crescents in diabetes may occur in diabetes in the absence of a secondary etiology and are composed of a mixture of parietal epithelial cells and visceral podocytes. Cells coexpressing parietal epithelial and podocyte markers suggest that parietal epithelial cells may transdifferentiate into podocytes in response to severe glomerular injury.


## 1. Introduction

Cellular crescents are typically associated with various forms of rapidly progressive glomerulonephritis (RPGN) such as antiglomerular basement membrane (GBM) disease, pauci-immune glomerulonephritis, and lupus nephritis [1,2]. A diagnosis of crescentic glomerulonephritis is typically treated aggressively with potent immunosuppressive agents.

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Crescents, however, may also be observed in other conditions. They have been documented in a wide variety of glomerular diseases including IgA nephropathy and Alport syndrome [2,3]. Rare case reports have described crescents in patients with diabetic nephropathy [4-6]. This finding, however, has received little attention in the published literature to date.

Glomerular crescents appear to be a ubiquitous response to glomerular injury. In RPGN, the inflammatory response results in GBM rupture, fibrin leakage into Bowman space, and crescent formation [2]. Such crescents typically contain inflammatory cells and fibrin within the proliferating epithelial cells. GBM rupture, however, may not be essential for crescent formation [7]. The nature of cells comprising crescents is an actively debated subject [8]. Some investigators identified cells of podocyte lineage within glomerular crescents [9-12]. Previous studies in mice suggested that visceral podocytes form bridges between the GBM and the parietal basement membrane (PBM) in early stages of crescent formation, perhaps triggering parietal epithelial cell (PEC) proliferation [7]. More recent studies using a transgenic mouse model of selective PEC depletion demonstrated that remaining PECs proliferate, leading to crescent formation [13]. Investigators have argued that PECs may transdifferentiate into podocytes, serving as a reservoir capable of replenishing damaged glomeruli [14-17].

The study of glomerular crescents has largely been limited to examination of human biopsies from patients with RPGN or animal models of glomerulonephritis. There are only rare reports describing crescent formation outside these scenarios [3-6]. When confronted with a biopsy containing crescents, it is critical to distinguish a pseudocrescent from the more aggressive inflammatory crescents associated with RPGN. Such a distinction is important to determine the appropriate treatment.

In the current study, 2 cases of diabetes with crescent formation were examined. Although crescent formation in diabetes is rare, their presence raises the suspicion for a coexisting disease such as pauci-immune glomerulonephritis. Distinguishing pseudocrescents associated with diabetes from true inflammatory crescents is essential to exclude this possibility. Comparing the nature of cells comprising diabetic crescents with the cells of inflammatory crescents may provide insights into the mechanism of glomerular injury in diabetes and provide pathologists with techniques to distinguish these entities. This is the first study to date to investigate the nature of crescent formation in diabetic glomeruli.

The protein claudin 1 is expressed exclusively in parietal epithelial cells [18]. In contrast, nephrin is expressed exclusively in podocytes [19,20]. Using antibodies to these 2 proteins, we investigated the cellular composition of diabetic crescents and compared the expression profile with cases of diabetes without crescents, inflammatory crescents, and normal-appearing glomeruli. Additional examination of the crescents using electron microscopy was performed.

## 2. Materials and methods

All cases were selected from the archives of the Lauren V. Ackerman Laboratory of Surgical Pathology at Washington University School of Medicine in St Louis, MO. Human studies were approved by the internal review board for human studies of Washington University School of Medicine. Renal biopsies from patients with diabetic nodular glomerulosclerosis with crescents (n = 2), diabetes with no crescents (n = 5), crescentic glomerulonephritis with inflammatory crescents (n = 5), and control kidneys from patients with minimal change disease (n = 1) and uninvolved kidney adjacent to resected renal tumors (n = 2) were stained for nephrin and claudin 1.

### 2.1. Immunofluorescence

Immunofluorescence staining was performed on formalin-fixed, paraffin-embedded tissue (3 $\mu$m). Following deparaffinization, the slides were subjected to antigen retrieval using 10 mM sodium citrate, 0.05% Tween20, pH 6.0 for 15 minutes. All primary antibodies were applied at 4°C overnight. Samples were probed with a Cy3 conjugated anti-sheep (no. 713-165-147; Jackson ImmunoResearch Lab, Inc) or Alexa Fluor 488 conjugated anti-rabbit secondary antibodies (no. 711-545-152; Jackson ImmunoResearch Lab, Inc., West Grove, PA) for 1 to 3 hours followed by bisbenzamide (Sigma, St. Louis, MO) staining. The primary antibodies used were a commercial sheep polyclonal anti-nephrin antibody (1:20, no. AF4269; R&D systems, Minneapolis, MN) and a rabbit polyclonal anti–claudin 1 antibody (1:200, no. AB15098; Abcam, Cambridge, MA).

### 2.2. Imaging

A Nikon 80i upright microscope with an attached Nikon C-1 Confocal system (Nikon, Melville, NY) was used to capture the immunofluorescence images. The images were analyzed using Nikon Elements software (Nikon). Z-stack immunofluorescence confocal images were used to count labeled cells in the glomerular tuft, lining the PBM and in the crescents. Cell counts were compared between groups using analysis of variance statistical analysis. An Olympus BX51 microscope equipped with an Olympus DP 71 camera was used to capture light microscopic images.

### 2.3. Clinical data

Demographic and clinical data are presented in the Table. Briefly, the diabetic renal biopsies with crescents were from 2 African American men ages 43 and 25 years with an 18-year history of diabetes mellitus type II and type I, respectively. The first patient was hospitalized for pneumonia and treated with vancomycin. His highest vancomycin trough was 20. During his hospital stay, his serum creatinine