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Commentary

Crescentic glomerulonephritis, a complication of severe immune glomerular injury, is the pathological correlate of rapidly progressive glomerulonephritis, mediated by both humoral and cellular effectors. In the current issue of the *JCI*, Chen et al. have implicated Bowman's capsule in functionally isolating potentially immune effectors, specifically antigen-specific CD8⁺ T lymphocytes, from podocytes. They suggest that, in crescentic glomerulonephritis, immune-mediated glomerular endothelial injury results in inside-out injury to the glomerulus, with subsequent leukocyte migration through a weakened or ruptured Bowman's capsule, resulting in outside-in injury. Effector T cells then recognize nephritogenic antigens presented by podocytes or other cells within the urinary space, enhancing injury and crescent formation.

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CD8+ cells and glomerular crescent formation: outside-in as well as inside-out

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Crescentic glomerulonephritis, a complication of severe immune glomerular injury, is the pathological correlate of rapidly progressive glomerulonephritis, mediated by both humoral and cellular effectors. In the current issue of the *JCI*, Chen et al. have implicated Bowman's capsule in functionally isolating potentially immune effectors, specifically antigen-specific CD8+ T lymphocytes, from podocytes. They suggest that, in crescentic glomerulonephritis, immune-mediated glomerular endothelial injury results in inside-out injury to the glomerulus, with subsequent leukocyte migration through a weakened or ruptured Bowman's capsule, resulting in outside-in injury. Effector T cells then recognize nephritogenic antigens presented by podocytes or other cells within the urinary space, enhancing injury and crescent formation.

Crescent formation in glomerulonephritis

The most severe and rapidly progressive forms of glomerulonephritis are characterized by the presence of glomerular crescents outside the glomerular capillary tuft within the urinary space. These lesions consist of fibrin, proliferating epithelial cells, macrophages, and CD4+ and CD8+ T lymphocytes. Without treatment or resolution of the underlying cause, the crescents first become fibrocellular, then fibrous. Subsequently, the glomerular tuft becomes obsolescent, with no chance of repair or recovery. While crescentic lesions are occasionally seen in nonimmune pathologies, glomerular crescents are typically a complication of several types of autoimmune glomerulonephritis. Crescent formation indicates severe and intense nephritogenic immunity, and people with crescentic glomerulonephritis are often prescribed significant immunosuppression to limit local inflammation and modify the underlying pathological immune response (1). Glomerular cres-

cents are most frequently found in the small-vessel vasculitides, Goodpasture's disease, and antineutrophil cytoplasmic antibody–associated (ANCA-associated) vasculitis, in which the autoantigens involved are either intrinsic to the kidney or initially present on neutrophils, then deposited in the kidney (2–4). Crescent formation can also complicate some cases of immune complex glomerulonephritis, including lupus nephritis, IgA nephropathy, and postinfectious glomerulonephritis. In addition to the formation of glomerular crescents and areas of segmental necrosis within the glomerular tuft, the most severe lesions also feature rupture of Bowman's capsule (5). This basement membrane is lined by parietal epithelial cells and normally contains the glomerular tuft and the urinary space (Figure 1, left panel). In a variety of inflammatory states, renal mononuclear phagocytes and T cells can be found adjacent to, but outside, Bowman's capsule, and in human and experimental crescentic glomerulonephritis, more severe lesions with more

leukocytes are observed when Bowman's capsule has ruptured (6).

The function of the glomerulus as a high-flow, high-pressure filter means that it is vulnerable to the full range of mediators that induce pathological inflammation (7). Humoral immunity has long been associated with glomerulonephritis. Despite the importance of cellular effectors in other autoimmune diseases, the role of effector T cells in severe autoimmune glomerulonephritis is less well defined, but effector CD4+ and CD8+ cells have been shown to participate in glomerular injury (8, 9). In humans, intrarenal CD8+ cells correlate with outcomes and CD8+ cell molecular signatures denote a poorer prognosis (4, 10, 11). Furthermore, in both autoimmune and nonautoimmune models of glomerulonephritis, depletion of CD8+ T cells limits histological and functional injury and transfer of CD8+ T cells can induce injury (12–14).

## Role of CD8+ cells in crescentic glomerulonephritis

In this issue of the *JCI*, Chen et al. shed light on the role of CD8+ cells in crescentic glomerulonephritis. They provide evidence for the ingress of periglomerular CD8+ T cells through localized breaches in Bowman's capsule, to subsequently participate in glomerular crescent formation and markedly amplify glomerular injury (15). Mice expressing the model antigen EGFP on podocytes received EGFP-specific CD8+ cells from T cell receptor (TCR) transgenic mice, evocatively named just EGFP death inducing (Jedi) mice. This model system required a separate and additional immunological trigger, as even when activated, as are T cells in autoimmune renal disease, usually via systemic exposure to autoantigen, these cells do not recognize EGFP on podocytes within normal glomeruli, potentially due to a lack of physical colocation. However, when injury to the elements of the glomerular filtration barrier is induced by the injection of antirenal basement
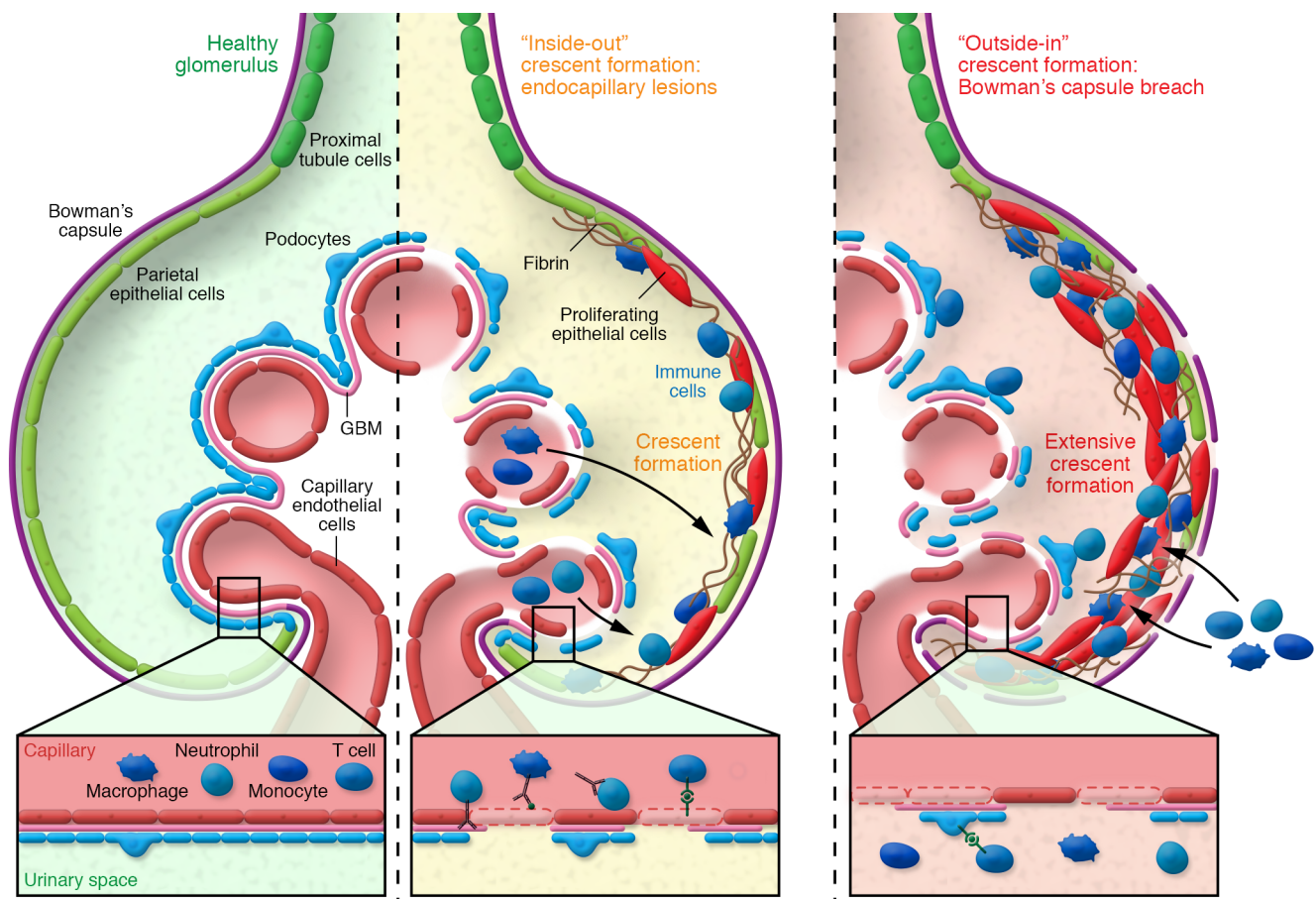
Figure 1. Schematic diagram summarizing inside-out and outside-in elements of glomerular crescent formation. Left panel: The basic structure of a healthy glomerulus consisting of the Bowman's capsule lined with parietal epithelial cells and the glomerular filtration barrier composed of endothelial cells, the glomerular basement membrane (GBM), and podocytes. Podocytes line the GBM with foot processes that wrap around the glomerular capillaries. Middle panel: Examples of some of the inside-out mechanisms that can cause endocapillary damage and early crescent formation following glomerulo-nephritis. Inset, left to right, neutrophils bind to autoantibodies in the GBM, as in anti-GBM glomerulonephritis. Antibodies bind to antigens lodged in the glomerulus with subsequent macrophage recruitment. ANCA activates neutrophils and mediates their recruitment to the glomerulus. CD8+ T cells can recognize antigenic peptides, potentially presented by endothelial cells. Right panel: Chen and colleagues describe the outside-in mechanism in which the antigen-specific CD8+ T cells, along with macrophages, located outside the glomerulus migrate into the urinary space following a breach in Bowman's capsule. Within the urinary space, CD8+ cells recognize antigens presented on podocytes, exacerbating injury and crescent formation.

membrane globulin (also known as neph-rotoxic serum), the Jedi effector T cells are highly pathogenic. These CD8+ cells recognize their cognate antigen on podo-cytes, resulting in podocyte loss, epithelial and local CD8+ infiltration and prolifera-tion with enhanced crescent formation, and functional kidney injury. The study also examined 2D biopsy sections from human crescentic glomerulonephritis and showed that CD8+ cells were more com-monly found in glomeruli in which Bow-man's capsule was ruptured. The authors present compelling correlative evidence, though the relation between Bowman's capsule rupture and crescent formation remains an association. Although chal-lenging, further studies using in vivo dual photon microscopy may be able to directly

visualize leukocytes eroding or breaking through Bowman's capsule.

How does one reconcile the promi-nence of crescent formation in endocapil-lary focused forms of rapidly progressive glomerulonephritis with the studies of Chen et al. that used a model podocyte antigen? Most intrarenal antigens current-ly known to be involved in human crescen-tic glomerulonephritis are located in or around the glomerular endothelium. The prototypic podocyte antigen in glomeru-lonephritis is the phospholipase A2 recep-tor 1 (PLA2R1), which causes the majority of cases of membranous nephropathy, a disease only uncommonly complicated by glomerular crescent formation (16). Experimentally, podocytes not only express MHC class I (MHC-I) and MHC-II,

but can act to present antigen to effector T cells. On the other hand, in ANCA-associ-ated vasculitis, which is commonly associ-ated with crescent formation, key autoan-tigens, such as myeloperoxidase (MPO), are found not only on neutrophils, in serum, and around the endothelium, but also subepithelially (3, 4, 17, 18). Thus, the capacity of podocytes to present antigen and the presence of autoantigens in and around podocytes after endothelial injury link endocapillary injury to podocytes and justify Chen et al.'s use of a podocyte anti-gen in their studies (15).

An integrated model of crescent formation
The answer to the question of how to incorporate the findings of Chen et al.

into an integrated model of crescent formation may come from examining previous experimental studies. While several experimental systems have used cells from TCR transgenic mice in experimental glomerulonephritis, arguably the most instructive have been two studies examining effector responses to ovalbumin (OVA) as a model antigen, using CD4^{+} OVA–specific OT-II cells and/or CD8^{+} OVA–specific OT-I cells (19, 20). In the first study, expressing OVA specifically on podocytes and transferring OVA-specific CD4^{+} and CD8^{+} cells resulted in significant proteinuria and OVA and dendritic cell–mediated accumulation of leukocytes in periglomerular and tubulointerstitial areas (20). Initially, there was little glomerular injury aside from signs of activated parietal cells, but at a later stage, after transfer of cells on several occasions, focal and segmental glomerulosclerosis was present and OVA-expressing cells were observed near Bowman's capsule. In the second system, OVA was planted intravascularly on and around the endothelium (19). Transfer of either Th1 or Th17 OT-II cells induced significant endocapillary glomerular injury, with early crescent formation after Th1 cell transfer, likely via intravascular antigen recognition (21). The studies of Chen et al. might be considered a composite of the Summers and the Heymann studies (19, 20). The initial injury in this dual stimulus model of nephrotoxic serum nephritis is induced by an antirenal basement membrane globulin preparation, which in its early days, models endocapillary inflammation mediated by in situ immune complex formation (22). This initial injury allows leakage of plasma proteins and glomerular antigens into the urinary space. In more severe forms of immune endocapillary injury, fibrin and other proteins induce leukocyte chemotaxis and accumulation, podocyte bridging and dedifferentiation, and parietal cell and renal progenitor cell activation, often resulting in the formation of early glomerular crescents (23–25). Combined with these inflammatory events in the urinary space, T cells and macrophages located outside the glomerulus then migrate through breaks in Bowman's capsule, where antigen-specific cells in the Jedi mouse model mount a significant immune attack and promote crescent formation (Figure 1 summarizes these concepts).

Future directions

With the studies of Chen et al., there is now more evidence for a two-step process, inside-out, then outside-in, in the development of crescentic glomerulonephritis, the pathological correlate of the feared clinical presentation of rapidly progressive glomerulonephritis. The studies further support a role of cell-mediated delayed-type hypersensitivity–like effector responses and suggest that further focus on selectively inhibiting cell-mediated immunity in these severe and often difficult to treat diseases is warranted.

Acknowledgments

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