Modulators of Crescentic Glomerulonephritis

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ABSTRACT

Glomerular crescent formation is a prominent feature of aggressive forms of glomerulonephritis and is associated with a poor prognosis. An understanding of the mechanisms involved in crescent formation is crucial for the development of new therapies for this disease. This article reviews current ideas on the pathogenesis of glomerular crescent formation and describes methods for modulation of this process. Emphasis is given to the role of the proinflammatory cytokines interleukin-1 and tumor necrosis factor-α in crescent development and its modulation by cytokine blockade.

Key Words: interleukin-1, tumor necrosis factor-α, cytokine, therapy

PATHOGENESIS OF CRESCENT FORMATION

Examination of biopsy specimens from patients with crescentic glomerulonephritis show heterogeneity in both the percentage of crescents and the stages of crescent development between glomeruli. Crescents can be divided into two main types: (1) cellular crescents, which contain cells with little or no collagen deposition within Bowman's space; and (2) crescents with varying degrees of fibrous organization, which are described as either fibrocellular or fibrous, depending on the balance of cells and fibrosis within Bowman's space. It is thought that cellular crescents represent the first stage of crescent formation, which can subsequently develop fibrous organization (5). This later step is thought to occur by the migration of periglomerular fibroblasts into Bowman's space through areas of disruption of Bowman's capsule (6,7). A schematic model of glomerular crescent formation is shown in Figure 1.

Cellular Crescents

Cellular crescents are defined as the presence of two or more layers of cells within Bowman's space. However, the composition of cellular crescents has been a controversial issue. Some studies have identified epithelial cells as the main component, whereas other investigations have demonstrated that macrophages are the major cell type within cellular crescents (8–13). These results suggest that cellular crescents are highly heterogeneous, with marked differences seen in various disease categories and also within individual kidneys.

Humoral reactants. Deposition of fibrin and plasma-derived fibronectin within Bowman's space is often associated with cellular crescent formation. The importance of fibrin deposition has been shown by the ability of anecrod and streptokinase treatment to prevent crescent formation in rabbit anti-glomerular basement membrane (anti-GBM) glomerulonephritis (14). Procoagulant activity of infiltrating glomerular macrophages is also crucial in fibrin deposition within Bowman's space (15). Deposition of plasma fibronectin within Bowman's space is thought to facilitate cell proliferation and accumulation during crescent formation (16).

Reversibility of cellular crescents. Not all crescents become fibrotic. As shown schematically in Figure 1, cellular crescents can either progress or resolve. Resolution of crescent formation is seen in the spontaneous recovery of a subgroup of patients with rapidly progressive glomerulonephritis (reviewed in Reference 17). Similarly, 90% of glomeruli exhibited cellular accumulation or exudate material within Bowman's

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space shortly after the induction of anti-GBM glomerulonephritis in the rabbit. However, only 50% of glomeruli progressed to a fibrotic stage, with the rest recovering a relatively normal appearance (18). This raises the question of what factors affect whether a cellular crescent progresses to a fibrotic stage or resolves. We know little of the mechanisms that determine the fate of cellular crescents, however, one factor that may influence the progression or resolution of cellular crescents is the structural integrity of Bowman's capsule. Disruption of Bowman's capsule is a common feature of glomeruli with fibrotic crescents and morphological studies have suggested that periglomerular fibroblasts and leukocytes enter Bowman's space through breaks in the basement membrane, leading to irreversible fibrosis of cellular crescents (6,7). A second and possibly related factor is macrophage accumulation within Bowman's space. A study of human crescentic glomerulonephritis found that cellular crescents containing predominantly epithelial cells rarely exhibit Bowman's-capsule rupture, whereas those containing macrophages were associated with rupture (13). In addition, spontaneous resolution of epithelial crescents has been described in rat anti-Thy-1 nephritis (19). Similarly, in a study of the progression of rat anti-GBM glomerulonephritis, cellular crescents containing primarily epithelial cells showed no evidence of Bowman's capsule rupture—even at late time points in the disease course—whereas cellular crescents containing primarily macrophages displayed an increased incidence of Bowman's capsule rupture as the disease progressed (20). Although there appears to be a good correlation between Bowman's-capsule rupture and fibrous de-
development of glomerular crescents, there is no direct evidence of a cause-and-effect relationship to support this postulate. Other possible mechanisms determining the fate of cellular crescents may be the nature of the chemotactic molecules, inflammatory cytokines, and prosclerotic growth factors produced by crescent cells or resident glomerular cells, such as parietal epithelial cells.

**Macrophage accumulation in Bowman's space.** The primary feature in the development of advanced cellular crescents is the large accumulation of macrophages within Bowman's space. One mechanism of macrophage accumulation within Bowman's space is migration of macrophages from the glomerular tuft, a process that is likely to involve chemotactic molecules and cell-matrix and cell-cell adhesion interactions.

Fibrin is the only chemotactic molecule directly implicated in macrophage accumulation in Bowman's space. However, several other molecules may be involved, such as monocyte chemoattractant protein-1 (MCP-1), macrophage colony stimulating factor, and macrophage migration inhibitory factor. Production of MCP-1 within crescents has been demonstrated by immunostaining in human glomerulonephritis (21). Also, each of these chemotactic molecules can be synthesized by proximal tubular cells, indicating that they might be produced by parietal epithelial cells during crescent formation (22-24).

Macrophages express a variety of adhesion molecules, such as VLA-4 and Mac-1 of the integrin family, that facilitate adhesion to fibronectin and fibrinogen—two molecules often present in Bowman's space at the start of crescent formation. In addition, VLA-4 and LFA-1 mediate adhesion to cells expressing VCAM-1 and ICAM-1, respectively. There is strong upregulation of VCAM-1 and ICAM-1 expression by parietal epithelial cells in most forms of glomerulonephritis, which may play a role in the accumulation of macrophages in Bowman's space during crescent formation (25). Indeed, administration of anti-ICAM-1 or anti-LFA-1 antibodies not only inhibits the induction of rat autoimmune anti-GBM glomerulonephritis, but can intervene in established disease and prevent further crescent formation, demonstrating a pathogenic role for the LFA-1/ICAM-1 interaction in this disease model (26).

Another adhesion molecule that may be involved in macrophage accumulation within Bowman's space is CD44. This cell-surface glycoprotein mediates adhesion to hyaluronic acid and is expressed by macrophages and a variety of other cell types (27). The combination of *de novo* CD44 expression by parietal epithelial cells and hyaluronic acid deposition in Bowman's space in rat anti-GBM glomerulonephritis may facilitate the migration and accumulation of CD44+ macrophages within Bowman's space through macrophage-matrix and hyaluronic acid-dependent macrophage-epithelial cell interactions (28,29).

**Local macrophage proliferation within Bowman's space.** Migration from the glomerular tuft is one route of macrophage accumulation within crescents. A second possibility is that macrophages proliferate locally within Bowman's space. Macrophage proliferation within cellular crescents in rat anti-GBM glomerulonephritis has been shown by the presence of mitotic figures, bromodeoxyuridine incorporation, and expression of the proliferating cell nuclear antigen by ED1+ macrophages. In one study, the number of proliferating macrophages within Bowman's space gave a significant positive correlation with the total number of macrophages within Bowman's space, but a negative correlation with the total number of macrophages within the glomerular tuft, suggesting that macrophage proliferation occurred locally within Bowman's space (Lan Hy, Nikolic-Paterson DJ, Mu W, Atkins RC, unpublished observations). If local proliferation were to prove to be a general mechanism of macrophage accumulation in crescentic glomerulonephritis, this would be a target for future drug development.

**T cells in crescents.** Although glomerular macrophage accumulation is evident in many forms of human glomerulonephritis, significant glomerular T cell infiltration is usually restricted to rapidly progressive glomerulonephritis (30). In a study of immunoglobulin (Ig) A disease, glomerular infiltration of immune-activated (IL-2 receptor-positive) T cells was prominent in "active" crescentic glomerulonephritis and correlated with the loss of renal function. Furthermore, the presence of glomerular T cells was associated with extensive disruption of Bowman's capsule and suggested the participation of a delayed-type hypersensitivity mechanism in crescent formation (31).

The importance of T cells in crescent formation has been demonstrated in animal models of glomerulonephritis. In a rat model of immune cell-mediated renal injury induced by immunization with the hapten azobenzeneboronic acid, followed by planting of the antigen in the kidney, glomerular crescent formation and Bowman's capsule rupture was observed in association with granulomatous inflammation (32). Similarly, an active model of crescentic glomerulonephritis in WKY rats induced by immunization with GBM was inhibited by blocking T cell activation. This was achieved by administration of CTLA-4-Ig, which blocks the CD28/B7 co-signal for T cell activation during antigen presentation. Indeed, when administration of CTLA-4-Ig was delayed until disease was established, there was still some reduction in the severity of crescent formation (33). In addition, the use of monoclonal antibodies to deplete T cells has demonstrated the requirement for CD4+ T cells in the induction of glomerular injury and crescent formation in murine lupus nephritis and rat accelerated anti-GBM glomerulonephritis (34,35).

**Disruption of Bowman's capsule.** The presence of interstitial collagens within Bowman's space is a prominent feature in the fibrous organization of cellular crescents. This is generally thought to be the result of interstitial fibroblasts entering Bowman's space.
through ruptured areas of Bowman’s capsule (6,7). Indeed, in rabbit anti-GBM glomerulonephritis, collagen deposition occurs in the periglomerular area in association with leukocyte infiltration before deposition is seen within the glomerulus (36). However, interstitial collagen has been noted within glomerular crescents in human glomerulonephritis in the absence of Bowman’s-capsule rupture, indicating that resident glomerular cells such as podocytes can produce interstitial collagens under some circumstances (37).

Periglomerular leukocytic infiltration is a common feature of aggressive forms of glomerulonephritis and there is evidence to implicate these cells in the disruption of Bowman’s capsule. In rat anti-GBM glomerulonephritis, focal infiltrates of immune-activated T cells and macrophages are invariably associated with Bowman’s-capsule rupture. Indeed, the presence of T cells within Bowman’s space is only apparent in those glomeruli with disruption of the capsule (20). These findings suggest that Bowman’s capsule is damaged through a delayed-type hypersensitivity mechanism, consistent with studies demonstrating the T cell dependence of crescentic glomerulonephritis described above.

The association of periglomerular leukocytes and Bowman’s-capsule rupture raises the question of whether capsular damage is mediated by crescent cells or periglomerular leukocytes. In rat anti-GBM glomerulonephritis, 26 to 52% of glomeruli with ruptured Bowman’s capsule had no evidence of crescent formation, suggesting that periglomerular—but not crescent—cells are essential for Bowman’s-capsule rupture (20). This observation suggests the intriguing possibility that some crescents may develop secondary to Bowman’s-capsule rupture, allowing the entry of macrophages, T cells, and fibroblasts into Bowman’s space. Indeed, it has been proposed that migration through tears in Bowman’s capsule is the major route of leukocyte entry into Bowman’s space during crescent formation (13).

Fibrocellular Crescents

Fibrocellular crescents are characterized by the presence of fibroblasts and collagen deposition within Bowman’s space. Numerous macrophages are still evident within Bowman’s space at this stage, whereas T cells are also seen in fibrocellular crescents. Both T cells and fibroblasts are thought to enter Bowman’s space from the periglomerular area through gaps in Bowman’s capsule. There is a gradual transition from a fibrocellular to a fibrous crescent phenotype, which involves the proliferation of fibroblasts and deposition of large amounts of collagen and other matrix molecules such as fibronectin. Fibroblast proliferation is likely to be driven by local production of growth factors such as acidic and basic fibroblast growth factors (FGF-1 and FGF-2). Combined immunohistochemistry and in situ hybridization studies have demonstrated FGF-receptor expression by fibroblast-like cells within fibrocellular crescents in rat crescentic glomerulonephritis. In addition, FGF-1 and FGF-2 mRNA and protein is expressed by several cell types—parietal epithelial cells, macrophages, and fibroblasts—within these crescents (38). Transforming growth factor β (TGF-β) is likely to play an important role in the deposition of collagen in Bowman’s space (39). Indeed, the presence of active TGF-β in urine is associated with glomerular scarring in crescentic glomerulonephritis in the rabbit (40).

Fibrous Crescents

Progressive collagen deposition within Bowman’s space leads to the development of a relatively acellular matrix structure akin to that seen in scar formation. The gradual loss of macrophages, T cells, and fibroblasts from Bowman’s space seen during the scarring process may be mediated by apoptosis (41). By this stage, the glomerular tuft is substantially compressed with marked capillary obliteration and is relatively acellular. The fibrotic process can progress further until the remaining capillary tuft is completely obliterated, resulting in global glomerular sclerosis.

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antagonist (IL-1ra) that competes with both IL-1α and β isoforms for receptor binding, and soluble forms of the IL-1 receptor. Similarly, TNF-α activity can be blocked by neutralizing antibodies and soluble forms of the TNF-α receptors (42,43). Using these reagents, a pathogenic role for IL-1 and TNF-α has been demonstrated in a number of animal models of inflammatory disease. Although in vitro studies have identified functional redundancy between IL-1 and TNF-α, this has yet to be established in animal models of disease.

**IL-1 in Crescentic Glomerulonephritis**

Glomerular IL-1 production has been demonstrated in human and experimental crescentic glomerulonephritis, with infiltrating macrophages implicated as the main source of this cytokine (44–48). Administration of IL-1β exacerbates glomerular injury during the first 24 h of endotoxin-enhanced anti-GBM glomerulonephritis (49), whereas blocking endogenous IL-1 suppresses the induction of glomerular injury in some models of anti-GBM disease (50–52). Blockade of IL-1 activity over a 2-wk period by constant administration of the IL-1ra prevented a loss in renal function and inhibited crescent formation in spite of continued moderate proteinuria in rat anti-GBM glomerulonephritis (53). IL-1 promotes both glomerular, and particular interstitial, macrophage infiltration by upregulating ICAM-1 expression (51,54). In addition, IL-1 promotes glomerular cell proliferation, although whether this is direct action has yet to be determined (53).

The role of IL-1 in the progression of established crescentic glomerulonephritis was examined by delaying IL-1ra treatment until glomerular crescent formation was already established. Infusion of IL-1ra over Days 7 to 21 of rat anti-GBM disease restored renal function and prevented further crescent formation (55). Indeed, blockade of IL-1 activity had a profound effect on the phenotype of crescents. In untreated animals, crescents seen at Day 7 were mostly of the early epithelial phenotype, whereas they were virtually all of the advanced or fibrocellular type by Day 21. In contrast, those crescents still evident in rats receiving IL-1ra treatment over Days 7 to 21 were of a predominantly early epithelial phenotype, demonstrating that IL-1 is important in macrophage accumulation within Bowman's space during the progression of early to advanced and fibrocellular crescents.

**TNF-α in Crescentic Glomerulonephritis**

Glomerular production of TNF-α has also been described in human and experimental crescentic glomerulonephritis (45,46,56–58). Blockade of endogenous TNF-α has been shown to suppress the induction of glomerular injury in rat anti-GBM glomerulonephritis (50,52). In addition, daily administration of anti-TNF-α anti-serum for 8 days, starting from the time of injection of anti-GBM serum, in primed rats caused a significant reduction in urinary albumin excretion, glomerular necrosis, and fibrin deposition. However, the consequence of TNF-α blockade on glomerular macrophage accumulation was not determined and few crescents were seen in this disease model (59). These findings have been confirmed and extended in a recent study demonstrating the pathogenic role of TNF-α in rat anti-GBM crescentic glomerulonephritis (60). In this study, TNF-α activity was blocked by the daily administration of a soluble dimeric form of the p55 chain of the Type I TNF-α receptor (TNFbp) from the time of anti-GBM serum injection until rats were euthanized 10 days later. Compared with the saline control, TNFbp treatment prevented a fall in renal function, produced a 39% reduction in proteinuria (P < 0.05 versus saline treatment) and almost abolished glomerular crescent formation (4 ± 1.4 versus 24 ± 2.8% crescents with TNFbp and saline treatments, respectively; P < 0.001). This suppression of crescentic glomerulonephritis was the result of a marked inhibition of glomerular and interstitial macrophage infiltration and a reduction in glomerular cell proliferation (60).

Given that IL-1 and TNF-α Share many proinflammatory properties, it is important to determine whether the inhibition of both cytokines will provide an added benefit over blocking each individually. This was addressed in rat crescentic anti-GBM glomerulonephritis by treatment with IL-1ra alone, TNFbp alone, or IL-1ra plus TNFbp over Days 0 to 10. Each treatment prevented a loss of renal function and produced a similar degree of suppression of proteinuria (27%, 39%, and 28%, respectively; all P < 0.05 versus saline). Similarly, each treatment markedly inhibited glomerular crescent formation (85%, 83%, and 92% reduction, respectively; all P < 0.001 versus saline) and caused comparable reductions in glomerular leukocyte infiltration and proliferation, glomerular lesions, tubular atrophy, and interstitial fibrosis (60). These results indicate that IL-1 and TNF-α act through similar pathways in glomerulonephritis. One possible common pathway of action is through the transcription factor NF-kappaB. Both IL-1 and TNF-α are active NF-kappaB in vitro and many of the genes whose transcription is upregulated by IL-1 and TNF-α have NF-kappaB sites in their promoter regions (61–63). This is typified by the presence of NF-kappaB sites in the promoters of the genes encoding both IL-1 and TNF-α (64,65). Whatever the common pathways of IL-1 and TNF-α action are, the results of this study suggest that no added benefit will be gained by attempting to block both cytokines simultaneously in clinical studies of glomerulonephritis.

**FUTURE THERAPEUTIC STRATEGIES IN CRESCENTIC GLOMERULONEPHRITIS**

There has been a dramatic increase in our understanding of immune, inflammatory, and fibrotic disease processes. One major benefit of this understanding has been the identification of common molecular
and cellular mechanisms underlying what were once thought to be unique disease processes. Although relatively few specific immunosuppressive drugs have been tested in animal models of glomerulonephritis featuring crescent formation, there are many potential therapeutic targets in this disease as outlined in Table 1.

Fibrin deposition within Bowman’s space is known to facilitate crescent formation in animals models of disease (14). However, current anticoagulant therapies have been disappointing clinically, showing little if any benefit in terms of renal function. Recent evidence has pointed to the activation of tissue factor as the main mechanism of extravascular coagulation (66). Thus, strategies to inhibit tissue-factor activation by neutralizing antibodies or recombinant tissue-factor pathway inhibitor (TFPI) may be effective in crescentic glomerulonephritis (67–69).

Inhibiting the accumulation of macrophages and T cells within Bowman’s space, indeed within the kidney as a whole, is a clear therapeutic goal. As outlined in Table 1, there are several levels at which mononuclear cell accumulation within the kidney can be blocked by targeting chemotactic molecules, proinflammatory cytokines, leukocyte adhesion molecules, and growth factors driving local proliferation. Some of these approaches have proven successful in models of crescentic glomerulonephritis using agents such as monoclonal antibodies and recombinant receptors and receptor antagonists (26,35,53,59). Having established the efficacy of such approaches, effort will be put into seeking better ways of blocking these mechanisms in vivo. For example, local production of soluble cytokine receptors or receptor antagonists through targeted gene therapy is an attractive option, although many technical difficulties remain to be overcome in such as approach (70).

As systemic inhibition of mononuclear cell recruitment to sites of inflammation may not be feasible on a long-term basis, an alternative approach is to inhibit the activation of macrophages and T cells that have entered the kidney. A number of cytokines and cell-surface glycoproteins are known to play important roles in the activation of macrophages and T cells (Table 1; References 71 through 73). The importance of one such T cell costimulating molecule, CD28, in crescent formation has been established in a rat model of autoimmune glomerulonephritis (33). An alternative means of inhibiting mononuclear cell activation is to block the intracellular signals generated through cell-surface activation. Although this is a technically difficult proposition, it is very attractive because activation of transcription factors, such as NF-kappaB and NF-ATp, play a crucial role in macrophage and T cell activation and in coordinating many components of the inflammatory response (74–77). Indeed, one of the mechanisms by which corticosteroids exert their anti-inflammatory action is through blockage of NF-kappaB activation (78). Thus, specific inhibitors of NF-kappaB activation would be expected to potent immunosuppressants without many of the side-effects of corticosteroids.

As the entry of fibroblasts into Bowman’s space marks the point of irreversibility for cellular crescents, targeting of the fibroblast response could also be of benefit. This could take the form of blocking the action of growth factors that stimulate fibroblast proliferation within the crescent (FGF, PDGF) or growth factors that promote matrix deposition (TGF-β). The ability of decorin treatment to inhibit matrix deposition in experimental glomerulonephritis suggests that local de-

### Table 1. Modulation of glomerular crescent formation

<table>
<thead>
<tr>
<th>MODULATION</th>
<th>POTENTIAL STRATEGY</th>
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<tr>
<td>Inhibit Fibrin Deposition</td>
<td>Block extravascular coagulation (Inhibit tissue factor, administer TFPI)</td>
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<tr>
<td>Inhibit Recruitment of Macrophages and T Cells</td>
<td>Inhibit the synthesis, secretion, or receptor binding of IL-1 and TNF-α</td>
</tr>
<tr>
<td>Systemic depletion of T cells by monoclonal antibodies</td>
<td>Block the action of molecules chemotactic for macrophages (MCP-1 and M-CSF) and T cells (RANTES, MCP-1)</td>
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<tr>
<td>Inhibit Local Macrophage Proliferation</td>
<td>Block macrophage entry into the kidney by interrupting adhesion molecule interactions (selectins, integrins, Ig family, CD44)</td>
</tr>
<tr>
<td>Inhibit Macrophage and T Cell Activation</td>
<td>Block macrophage growth factors (M-CSF)</td>
</tr>
<tr>
<td>Inhibit Fibroblast Accumulation and Matrix Synthesis</td>
<td>Block growth factors that promote fibroblast proliferation (FGF, PDGF) and fibroblast matrix synthesis (TGF-β)</td>
</tr>
</tbody>
</table>

*TFPI, tissue-factor pathway inhibitor; IL, interleukin; TNF-α, tumor necrosis factor-α; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony stimulating factor; RANTES, regulated upon activation, normal T-expressed and presumably secreted; IFN-γ, interferon-gamma; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β.*
livery of decorin-expressing plasmids into the kidney could provide substantial protection against progressive fibrosis, including fibrous organization of cellular crescents (79).

SUMMARY

The importance of cellular immune mechanisms in the progression of glomerulonephritis, including crescentic disease, has become increasingly evident. The rapid advances in our understanding of the steps involved in the induction and execution of the cellular immune response has led to the development of a variety of highly specific immunosuppressive agents. In particular, the impressive ability of cytokine blockade to suppress experimental crescentic glomerulonephritis has opened the way for clinical trials using agents such as IL-1ra or the TNFβp as adjuncts to current therapies.

ACKNOWLEDGMENTS

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