Freedom isn't always free: immunoglobulin free light chains promote renal fibrosis

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Multiple myeloma (MM) is a relatively common hematologic malignancy, and up to half of patients with MM present with renal dysfunction at the time of diagnosis. MM-associated renal injury has been linked to an excess level of monoclonal immunoglobulin free light chains (FLCs) in the circulation; however, it is not clear how these FLCs drive renal pathology. In this issue of the *JCI*, Ying et al. unravel a novel mechanism by which FLCs mediate renal injury in MM by inducing fibrotic and inflammatory pathways in the kidney. Specifically, FLC-mediated production of H_2O_2 was shown to activate JAK2/STAT1 signaling, increase production of IL-1β via induction of capsase-1, and promote activation of TGF-β via αvβ6 integrin. Moreover, the authors identified a tryptophan residue within a specific monoclonal FLC that was required for optimal H_2O_2 production and downstream signaling. A better understanding of the drivers of MM-associated renal injury has potential for the identification of promising therapeutic targets.

Renal injury in multiple myeloma

Clonal plasma cell dyscrasias, including monoclonal gammopathy of undetermined significance (MGUS), solitary plasmacytoma, immunoglobulin-mediated amyloidosis, and multiple myeloma (MM), are relatively common disorders, with 2% to 3% of patients over the age of 50 affected by premalignant MGUS. MGUS can progress to MM, which accounts for 12% to 13% of all hematologic malignancies in the United States (1). The complications of MM include hypercalcemia, anemia, bone disease, such as lytic lesions and/or severe osteopenia, and renal insufficiency. MM is the most common malignancy associated with end-stage renal disease (2), and, depending on the diagnostic criteria (serum creatinine level), 20% to 50% of patients have renal failure at diagnosis (3). Because of the high prevalence of renal involvement in MM and its documented importance as a prognostic indicator (4), an understanding of the mechanisms that lead to the development of renal injury in MM are needed.

The principal factor that contributes to renal injury in MM is excess monoclonal immunoglobulin free light chains (FLCs), which can be toxic, resulting in damage of various renal structures, especially the tubules (3). Kappa (κ) and lambda (λ) FLCs are normally produced by the lymphoid system during immunoglobulin synthesis and are freely filtered by the glomerulus and catabolized in the proximal tubule. Within the proximal tubule, FLCs are endocytosed via a receptor-mediated mechanism that involves megalin and cubilin (5, 6) and then hydrolyzed, with the resulting amino acids returned to the circulation. Under normal conditions, the bulk of FLCs are catabolized, and the levels of FLCs present in urine are very low (1–10 mg/day). However, in the case of MM, the amount of circulating FLCs is markedly increased, and the proximal tubular cells are unable to catabolize all of the FLCs. Those FLCs that are not catabolized move to the distal tubule, where they interact with Tamm-Horsfall protein, leading to the development of cast nephropathy (7). The formation of casts is not present in every patient and depends on the nephrotoxicity of the specific FLC. A less common manifestation of MM is Fanconi syndrome, which occurs when FLCs undergo homotypic polymerization and form crystals within the endolysosomal system. In addition, the high rate of FLC endocytosis by the renal tubular cells in MM induces the production of inflammatory cytokines, including IL-6, IL-8, and TNF-α, via pathways involving NF-κB and MAPK activation (8, 9). The inflammatory and fibrotic pathways that are induced by the endocytosis of FLCs are the subject of current research.

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Linking FLCs to renal fibrosis

The renal inflammatory pathways that are triggered by FLCs have been shown to be dependent on the ability of these proteins to catalyze the formation of hydrogen peroxide (H_2O_2) from free water. This catalytic reaction was first shown for intact IgG molecules and Fab fragments by Wentworth et al. (10) and later for FLCs by Wang and Sanders (11). Similar to the differential effects of FLCs on the induction of cast nephropathy and Fanconi syndrome, distinct monoclonal FLCs differ in their abilities to catalyze H_2O_2 formation. In this issue, Ying et al. have identified a mechanism whereby monoclonal immunoglobulin FLCs activate inflammatory and fibrotic signaling pathways and show that this activation stems from the ability to generate H_2O_2 (12). Incubation of cultured human kidney epithelial cells with monoclonal preparations of κ or λ light chains promoted activation of JAK2 and STAT1. Further investigation revealed that monoclonal FLCs activate caspase-1 and increase production of IL-1β and active TGF-β. Activation of TGF-β also involved

Conflict of interest: The authors have declared that no conflict of interest exists.

Reference information: *J Clin Invest*. 2019;129(7):2660–2662. https://doi.org/10.1172/JCI129704.

the expression of αvβ6 integrin on kidney epithelial cells, which has been shown in other disease states (13). Ying and colleagues confirmed the link between the JAK/STAT signaling and H_2O_2 production by FLCs, as the addition of the H_2O_2 scavenger 1,3-dimethyl-2-thiourea to the media prevented JAK/STAT activation.

For many of the renal complications associated with MM, the structure of the FLC variable region (V_L) determines its ability to induce renal pathology. For example, FLCs that cause Fanconi syndrome are generally of the κ isotype and have nonpolar or hydrophobic residues in the complementarity-determining region (CDR) (14). Similarly, the CDR3 region determines binding to Tamm-Horsfall protein, thus demonstrating that certain monoclonal light chains are more likely to induce cast nephropathy in MM (15). Ying and colleagues generated a molecular model of a κ FLC V_L domain and discovered that a tryptophan (W) residue extends into the binding pocket. Using recombinant proteins, the authors elegantly showed that the recombinant V_L with the W residue produced more H_2O_2 than did the V_L with a W40G substitution. The recombinant V_L with the native sequence also increased STAT1 activation and promoted IL-1β and TGF-β production. The results of this study confirm the importance of amino acid sequence–specific effects of monoclonal FLCs on renal injury.

These in vitro findings were explored further in vivo. Specifically, Ying et al. injected *Stat1*$^{+/+}$ and *Stat1*$^{-/-}$ mice with a monoclonal κ2 light chain for 10 days. The κ2 FLC localized to the proximal tubule brush border in both *Stat1*$^{+/+}$ and *Stat1*$^{-/-}$ mice, but only induced albuminuria and increased urinary excretion of the tubular epithelial cell injury marker KIM-1 in *Stat1*$^{+/+}$ mice. The pathway leading to caspase-1 activation was also confirmed in vivo. While these studies were relatively short term and not extended to a time point at which the mice had elevated serum creatinine and declining renal function, they offer an important proof of concept supporting the cell culture studies. Importantly, these results support the idea that monoclonal FLCs in the proximal tubule promote a proinflammatory and profibrotic state that involves IL-1β and TGF-β early in disease progression. Although not shown in this study, mounting evidence points to a central role for IL-1β as a potent inflammatory mediator in the kidney that triggers cell stress and the expression of inflammatory mediators (16). Recently, IL-1β was also shown to regulate the metabolism of kidney stromal cells, thereby leading to the initiation of fibrosis (17). TGF-β has long been recognized as a fibrosis mediator; however, this cytokine can also induce protective and antiinflammatory effects (18). Taken together, the results of the study highlight a potentially important link between monoclonal FLCs and the initiation of renal inflammatory and fibrotic pathways in MM.

Clinical implications

The median survival for patients with MM is approximately three years (19), and renal impairment greatly reduces survival in this patient population. Clinical data suggest that a prompt reduction in circulating FLCs, either by the use of chemotherapeutic agents or direct removal from the plasma, can lead to renal recovery (20, 21). In particular, the proteasome inhibitor bortezomib is the most effective chemotherapeutic agent for treating myeloma with renal injury (22). Plasma cells synthesize and assemble large quantities of immunoglobulins, generate the bulk of circulating FLCs, and are highly sensitive to proteasome inhibition (23). In addition, bortezomib inhibits both NF-κB and MAPK pathways (24), which have been shown to act downstream of FLCs in the kidney. The effect of bortezomib in MM with renal involvement has been well documented (22), and it has also been effective in ameliorating renal injury in autoantibody-mediated diseases such as systemic lupus erythematosus (SLE) (25–27).

It should be noted that numerous autoimmune and inflammatory diseases, including SLE, rheumatoid arthritis, multiple sclerosis, and diabetes, feature elevated circulating polyclonal FLCs (28). The pathological significance of these polyclonal FLCs has not yet been fully explored, but it is likely that they contribute to renal injury in some of these diseases. Moreover, patients with chronic kidney disease (CKD) (approximately 14% of the population in the US) have impaired renal clearance of FLCs as their glomerular filtration rate decreases. Studies suggest that these polyclonal FLCs may also contribute to tubular injury in patients with CKD (29). Thus, the present work by Ying et al. is important, because it substantially expands upon previous knowledge related to the pathogenic role of monoclonal FLCs within the kidney that may ultimately provide insight for the development of novel therapeutics.

Acknowledgments

Work by the authors is funded by a Veterans Affairs Merit Award (BX002604-0 1A2, to MJR); a National Research Service Award from the National Heart, Lung, and Blood Institute (NHLBI), NIH (F32HL137393, to EBT); and the NIH (P01HL051971, P20GM104357, U54GM115428, and T32HL105324-05, to the Department of Physiology, University of Mississippi Medical Center).

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