Macrophage-derived TGF-β in renal fibrosis: not a macro-impact after all

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Macrophages are a group of fascinating immune cells that play a critical role in mediating kidney injury, repair, and fibrosis (9). The vast majority, if not all, of the macrophages originate from myeloid precursors in the bone marrow and are released into circulation as monocytes (10). They then infiltrate the kidney and display a unique spectrum of activities in multiple injury models including renal transplantation, unilateral ureteral obstruction (UUO), and ischemia-reperfusion (I/R) injury (3, 7, 9). Indeed, while macrophages are certainly capable of phagocytosis and antigen presentation, recent studies suggest diverse phenotypes and surprising plasticity throughout the course of disease. For instance, Lee et al. (2) showed that proinflammatory M1 phenotype macrophages are recruited into kidneys within the first 48 h after I/R injury. These cells promote renal injury, as macrophage depletion before injury prevents disease. In the recovery stage, macrophages switch to an alternatively activated anti-inflammatory M2 phenotype that promotes tubular cell regeneration and repair (2). However, this switch can be both helpful and harmful, as M2 macrophages can ultimately cause fibrosis as part of a wound-healing response (6). Since M2 macrophages generate significant amounts of the profibrotic cytokine transforming growth factor (TGF)-β, it has been believed that this might be at least one of the mechanisms by which macrophages drive the development and progression of fibrosis. This hypothesis remains difficult to test directly in vivo.

In an issue of the American Journal of Physiology-Renal Physiology, Huen et al. (1) utilize a unique Cre-lox system to examine the role of macrophage-derived TGF-β in kidney fibrosis. In this system, mice that are heterozygotes for a floxed TGF-β1 gene (the other allele is null for TGF-β1) are crossed with LysM-Cre transgenic mice that possess the Cre recombinase under control of a myeloid-specific promoter. As such, the crossbred mice are intended to be myeloid-specific knockouts for TGF-β1. It should be noted that LysM-Cre is not expressed consistently in all monocytes and can be prone to mosaic expression and interanimal variability (5). Nonetheless, the authors demonstrate that these knockout mice have an impressive 93% reduction in TGF-β1 expression in bone marrow-derived macrophages. In terms of kidney-derived macrophages, the reduction was a less impressive 64.5%. Despite this lower efficiency, phosphorylation of Smad2/3, the canonical intracellular signaling event after TGF-β ligation with the TGF-β receptors, was significantly decreased at 28 days after I/R compared with wild-type mice. At this late time point, there was a nonsignificant trend toward less fibrosis in these mice and a mild but significant reduction in collagen I and fibroblast-specific protein-1 expression. These studies were repeated in the UUO model, but with no apparent difference in phenotype.

These findings lead to a number of interesting inferences. First, if the less robust nature of TGF-β knockout in kidney macrophages is not due to technical issues related to the animal model, it may suggest that the kidney is infiltrated after I/R by a specific population of myeloid cells, many of which never expressed LysM during their development. Interestingly, this would indicate that some myeloid precursors are selected as early as during bone marrow development as being destined for the kidney. Alternatively, as new evidence suggests that not all macrophages originate from myeloid precursors, it is possible that some resident kidney macrophages may not express LysM (10).

Another discovery is that Smad2/3 phosphorylation/activation seems to rely on a very specific level of TGF-β production. The authors show that in heterozygote mice, which produce TGF-β mRNA at 50% of the level of wild-type mice, there is a nonsignificant trend toward decreased Smad2/3 phosphorylation 28 days after injury. Meanwhile, in the macrophage knockouts they demonstrate a 65% reduction in TGF-β mRNA, but this completely abolished Smad2/3 phosphorylation. While this observation is based on mRNA rather than protein levels, it suggests that a narrow window exists where Smad2/3 phosphorylation can be triggered despite a 50% reduction, but not stimulated when 65% reduction occurs. As the authors speculate, this indicates that TGF-β production is generally excessive for canonical signaling in wild-type mice exposed to injury. Also, the loss of Smad2/3 signaling in the knockouts indicates that macrophages are indeed a key source for TGF-β signaling activation at this time point.

The most interesting finding is that ablation of Smad2/3 phosphorylation 28 days after I/R in knockout mice had only a mild effect on renal fibrosis, the sine qua non of progressive chronic kidney disease. In UUO, there was no impact on fibrosis severity at all. This unexpected finding challenges our notions of the importance of the TGF-β-Smad2/3 pathway in renal fibrosis. It is not very likely that Smad2/3 phosphorylation is unimportant, because previous studies show diminished fibrosis in Smad3 knockout mice exposed to renal injury (8). In addition, studies that have blocked TGF-β signaling demonstrated protection from renal fibrosis (4). Therefore, it may be that nonmyeloid sources of TGF-β are sufficient to drive fibrosis before this time point. Furthermore, the exact temporal and spatial expression of TGF-β and Smad2/3 activation is probably more important than the total amount of Smad2/3 activation detected on a “whole kidney” level. Furthermore, upregulation of other TGF-β isoforms (which the authors show) and signaling through non-Smad signaling pathways (which was not assessed) could be the explanation for these findings and require future study.

So where does this leave us? With the preponderance of evidence from previous studies, we must continue to assume...
that TGF-β expression does play a major role in fibrosis development, as does the macrophage. However, the careful studies of Huen et al. presented herein provide important data demonstrating that macrophage-specific deletion of TGF-β1 is not sufficient to prevent disease. If macrophage-derived TGF-β1 is not important, it is the task of future studies to determine the exact sources of pathogenic TGF-β as well as the target cells responding to this signal after injury. And finally, the relevance of these findings to human chronic kidney disease must be assessed to determine the true clinical impact for our patients with kidney disorders.

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