Basophils orchestrate kidney fibrosis

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Research Highlight

Basophils orchestrate kidney fibrosis

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Abstract

Basophils, though represent a minor population of leukocytes, have diverse roles in various pathophysiologies. A recent report provides an additional evidence on the pathogenic role of basophils in promoting kidney fibrosis by secreting IL-6 and promoting the Th17 cell differentiation.

Basophils are rare granulocytes that take part in T helper (Th) type 2 immunity, principally through the secretion of interleukins IL-4 and IL-13. Besides their role in the defense against helminth infections, basophils play a major role in the pathogenesis of various allergic inflammatory diseases, autoimmune diseases, and cancer.[1] Basophils express a diverse array of receptors, and hence could sense signals from various sources including cytokines, toll-like receptor agonists, and allergens, and subsequently undergo rapid activation and release inflammatory mediators. In addition to IL-4 and IL-13, basophils produce histamine, prostaglandins, leukotrienes, IL-6 and chemokines in response to the stimuli. All these inflammatory mediators are potential contributors of basophil-mediated pathogenesis.

Fibrosis is characterized by the development of fibrous connective tissue in order to scar injury or tissue damage. Renal fibrosis (RF) is described as the altered kidney's ability to regenerate after injury leading to a gradual loss of renal function, potentially evolving to a life-threatening renal failure and a requirement for dialysis or kidney transplantation. Our comprehension of immune cell interactions that trigger RF is still unfolding. Previous experimental studies have suggested a possible role for basophils in mediating cardiac allograft fibrosis by triggering fibroblast activation, expanding myofibroblasts, promoting the production of extracellular matrix proteins and remodelling of the fibrotic organ.[2] Now, by applying single-cell sequencing in a murine model of KF, Katalin Susztak and colleagues report a mechanistic evidence on the pathogenic role of basophils in promoting fibrosis.[3]

To understand the pathogenesis of KF, Doke et al. compared the transcriptome profile of kidney of wild-type mice (C57BL/6) subjected to sham or unilateral ureter obstruction (UUO) surgery leading to fibrosis. They identified the genes enriched in basophil cluster in the fibrotic kidneys of UUO mice and further studied the unappreciated role of these granulocytes in RF (Fig. 1).[3]

Following subclustering analysis of the proximal tubules (PT) in the UUO kidneys, the authors described a subset of profibrotic PT cells that evolve from PT precursors and display an increased expression of genes encoding fibroinflammatory cytokines and chemokines. Doke et al. used *in silico* analysis and in situ hybridization to further analyze the cell interaction between the profibrotic PT cells and immune cells, and noted that fibrotic PT cells could contribute to the recruitment of $CXCR2^{+}$ basophils in UUO kidneys by secreting CXCL1.[3]

In order to prove the role of basophils in the pathogenesis of KF, Doke et al resorted to two complementary approaches of depletion of these cells in vivo by using either conditional $Mcpt8^{Cre}$/DTR mice or by injecting MAR-1 antibody that depletes $Fc\varepsilon RI\alpha^{+}$ cells (all basophils intensely express this molecule). In line with their hypothesis, basophil depletion led to the reduced expression of fibroblast markers *Col1a1*, *Col3a1*, *Timp1* and *Acta2* in UUO kidneys, and lessened the RF and severity of tubular interstitial damage.

How do basophils contribute to the pathogenesis of KF? Previous study in the chronic cardiac allograft rejection model has identified basophil-derived IL-4 as a major profibrotic cytokine.[2] However, subsequent study by the same group identified IL-3 as a key profibrotic cytokine that exerted profibrotic functions and organ remodelling, even if IL-4 was completely suppressed.[4] Mechanistically, IL-3 exerted profibrotic effects by

activating and inducing IL-6 in the infiltrating basophils. In line with this report, single-cell analysis and in situ hybridization identified an enhanced expression of *Il6* in the basophils of UUO kidneys. In addition, basophils of UUO kidneys displayed higher expression of the receptors (*Il18r1* and *Il1rl1*) for the cytokines IL-18 and IL-33 that are known to activate basophils. Bulk RNA-seq data from UUO kidneys also confirmed these results but no difference in the expression of *Il4* was observed between UUO and sham kidneys.[3] Additional investigations documented that stroma cells in UUO kidneys contribute to IL-18 and IL-33. *In vitro* basophil stimulation with IL-18 or IL-33 led to the induction of IL-6.[3] Further investigations on the signals that activate stroma cells are required.

It is well known that IL-6 supports the differentiation of Th17 subset of $CD4^+$ T cells.[5] Therefore, authors were aimed at pinpointing whether Th17 cells and their products are the further down-stream mediators of KF. The expression of IL-6 receptor transcripts were higher in $CD4^+$ T cells and Th17 cells from UUO kidneys, and RNA velocity analysis highlighted an enhanced differentiation of Th17 cells. Genetic depletion of basophils reduced Th17 cells and Th17 signatures in UUO kidneys.[3]

All these data together indicated that $CXCR2^+$ basophils migrate to the kidneys in response to CXCL1 secreted by profibrotic PT cells in UUO mice. These basophils undergo activation by stromal-secreted IL-18 and IL-33 and produce IL-6 that in turn supports Th17 response. Based on these findings and as a proof of concept, authors pre-treated mice with IL-6 receptor antagonists before inducing UUO. In line with the findings, IL-6 receptor antagonism reduced the expression levels of various fibrosis markers and the accumulation of collagen in the kidneys.[3]

Are these observations relevant for the humans? To address this, the authors used kidney samples from the patients with chronic kidney disease. It was noteworthy that basophil number, *IL6*, *CXCL1, IL18, IL33* and *IL17d* transcripts were strongly correlated with the degree of renal fibrosis,[3] thus validating the translational value of the experimental data.

Though basophils are implicated in the pathogenesis of several diseases, the signals that drive basophil activation vary. While basophil activation was induced by T cell-derived IL-3 in cancer progression[6] or in chronically rejecting allografts,[4] Doke et al described basophil activation via IL-18 and IL-33 in KF. On the other hand, thymic stromal lymphopoietin is implicated in eliciting basophil responses in eosinophilic esophagitis.[7] Human basophils though can be activated by IL-33, in contrast to mouse basophils, have been shown not to respond to IL-18 even though they constitutively express IL-18 receptors.[8] Moreover, IL-3 and IL-33 activate distinct signaling pathways in blood basophils suggesting different activation states or functions of cytokine-activated basophils in these pathologies.

While there are currently no drugs for fibrosis or chronic kidney disease that would specifically target the kidney, the study of Doka et al provides certain clues on how to improve KF by targeting either basophils, the CXCR2/CXCL1 axis that recruit basophils or IL-6, the down-stream mediator of KF. Blocking CXCR2 by an inhibitor (Reparixin) reduced neutrophil accumulation in the kidneys and maintained kidney function in a reperfusion injury model.[9] Another CXCR2 inhibitor Danirixin is currently being investigated in the clinical trials.[10] The same strategy could be used to reduce

the recruitment of basophils during KF (Fig.1). But redundancy in the chemokine axis is the main drawback. Though monoclonal antibody-based basophil depletion has been achieved in experimental models, it is too far from the human application. However, tocilizumab, a humanized monoclonal antibody against IL-6 receptor is already in the clinic since many years and this drug can be examined immediately in the chronic kidney disease. Tocilizumab treatment though may not alter the basophils or their activation, can reduce the number of Th17 cells as shown in rheumatoid arthritis.[11]

ADDITIONAL INFORMATION

Competing interests: Authors declare that they have no competing interests

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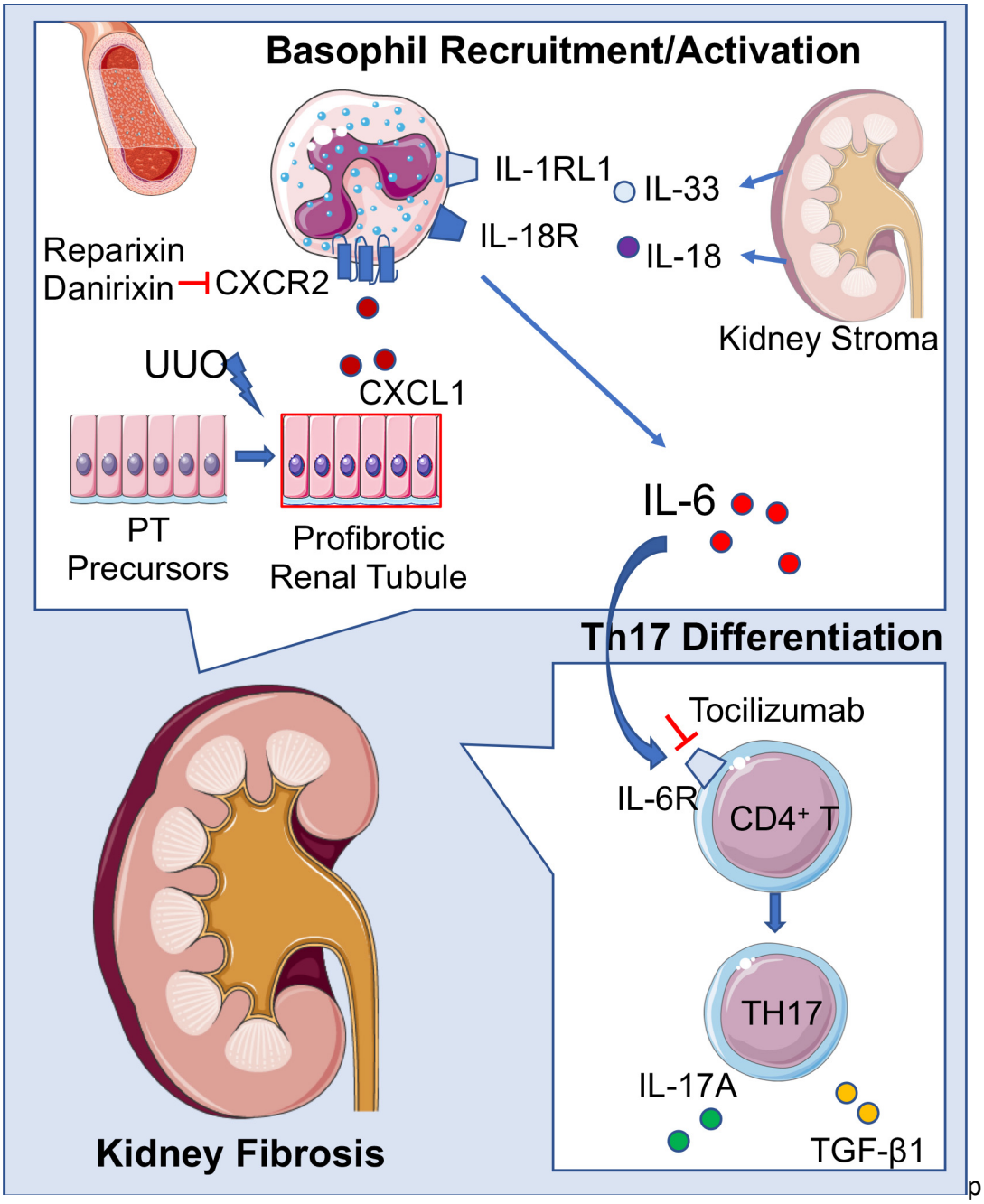
FIGURE LEGEND

Fig.1. Pathogenic role of basophils in kidney fibrosis and possible therapeutic interventions. During kidney fibrosis, basophils are recruited by profibrotic renal tubules via CXCR2/CXCL1 axis and undergo activation by kidney stroma produced IL-18 and IL-33. These activated basophils produce IL-6 that induces the differentiation of Th17 cells. IL-6 and Th17 cells act as down-stream mediators of kidney fibrosis. The pathogenic roles of IL-6 could be blocked by monoclonal antibodies that target IL-6 receptor (Tocilizumab). CXCR2/CXCL1 axis that recruit basophils could be pharmacologically targeted though redundancy in the chemokine system is the major draw-back. PT: Proximal tubules.


Basophil Recruitment/Activation
IL-1RL1
IL-33
IL-18R
IL-18
Reparixin
Danirixin
CXCR2
Kidney Stroma
UUO
CXCL1
PT
Precursors
Profibrotic
Renal Tubule
IL-6
Th17 Differentiation
Tocilizumab
IL-6R
CD4⁺ T
TH17
IL-17A
TGF-β1
Kidney Fibrosis

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