The IL-4 receptor α has a critical role in bone marrow–derived fibroblast activation and renal fibrosis

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Renal fibrosis is a common pathway leading to the progression of chronic kidney disease, and bone marrow–derived fibroblasts contribute significantly to the development of renal fibrosis. However, the signaling mechanisms underlying the activation of these fibroblasts are not completely understood. Here, we examined the role of IL-4 receptor α (IL-4Rα) in the activation of myeloid fibroblasts in two experimental models of renal fibrosis. Compared with wild-type mice, IL-4Rα knockout mice accumulated fewer bone marrow–derived fibroblasts and myofibroblasts in their kidneys. IL-4Rα deficiency suppressed the expression of α-smooth muscle actin, extracellular matrix proteins and the development of renal fibrosis. Furthermore, IL-4Rα deficiency inhibited the activation of signal transducer and activator of transcription 6 (STAT6) in the kidney. Moreover, wild-type mice engrafted with bone marrow cells from IL-4Rα knockout mice exhibited fewer myeloid fibroblasts in the kidney and displayed less severe renal fibrosis following ureteral obstructive injury compared with wild-type mice engrafted with wild-type bone marrow cells. *In vitro*, IL-4 activated STAT6 and stimulated expression of α-smooth muscle actin and fibronectin in mouse bone marrow monocytes. This was abolished in the absence of IL-4Rα. Thus, IL-4Rα plays an important role in bone marrow–derived fibroblast activation, resulting in extracellular matrix protein production and fibrosis development. Hence, the IL-4Rα/STAT6 signaling pathway may serve as a novel therapeutic target for chronic kidney disease.

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Chronic kidney disease (CKD) is a leading cause of death and a serious global health challenge. The prevalence of CKD continues to increase worldwide.[1] Therefore, new strategies for the prevention and treatment of CKD are much needed to reduce its morbidity and mortality. Renal fibrosis is a pathologic hallmark of CKD, which is manifested by extensive fibroblast activation. Activated fibroblasts produce a large amount of extracellular matrix (ECM), leading to the destruction of renal parenchyma and progressive loss of kidney function.[2–4] Despite improvement in the knowledge regarding diverse CKD-related aspects, the cellular and molecular events leading to the development of renal fibrosis and eventually to renal failure are not completely understood. Therefore, a better understanding of the pathogenesis of renal fibrosis is essential for developing novel therapeutic strategies for treating chronic fibrotic kidney disease and preventing its progression.

Traditionally, activated fibroblasts were considered to arise from resident fibroblasts.[5,6] However, recent accumulating evidence indicates that bone marrow–derived fibroblast precursors significantly contribute to the population of activated fibroblasts and the development of renal fibrosis.[7–10] Bone marrow–derived fibroblast precursors, termed as fibrocytes, are spindle-shaped cells that express hematopoietic markers, such as cluster of differentiation 45 (CD45) and CD11b, and mesenchymal markers, such as platelet-derived growth factor receptor-β (PDGFR-β) and vimentin.[11–13] Our previous studies have demonstrated that bone marrow–derived fibroblast precursors substantially contribute to the development of renal interstitial fibrosis.[13–16] However, the molecular mechanisms underlying the activation of these fibroblasts are not completely understood.

The activation of myeloid fibroblasts is regulated by cytokines that are locally produced by T helper (Th) cells. Th1 cells produce antifibrotic cytokines, such as interferon–γ, that suppress myeloid fibroblast differentiation, whereas Th2 cells produce profibrotic cytokines such as, interleukin (IL)-4 and IL-13, that promote myeloid fibroblast differentiation.[17–19] IL-4 receptor α (IL-4Rα) is a key component of the Th2 cytokine receptor, which mediates Th2 cytokine–signal transducer and activator of transcription 6 (STAT6) signaling pathway transduction and subsequent effector functions.[20–22]

We previously demonstrated that Th2 cytokines activate the Janus kinase 3 (JAK3)–STAT6 signaling pathway to stimulate the activation of myeloid fibroblasts, which play a crucial role in the development of renal fibrosis.[13] Therefore, we hypothesized that IL-4Rα mediates Th2 cytokine–STAT6 signaling transduction in myeloid fibroblast activation and renal fibrosis. Here, we investigated the role of IL-4Rα signaling in activating bone marrow–derived fibroblast precursors in murine models of renal fibrosis induced by folic acid (FA) or unilateral ureteral obstruction (UUO). Our results demonstrate that IL-4Rα deficiency inhibits the activation of bone marrow–derived fibroblasts and suppresses the development of renal fibrosis.

RESULTS

IL-4Rα deficiency impairs myeloid fibroblast accumulation

Myeloid fibroblast precursors substantially contribute to the pathogenesis of renal fibrosis.[6,13,14,23] To investigate the role of IL-4Rα in the activation of bone marrow–derived fibroblasts, wild-type (WT) and IL-4Rα knockout (KO) mice were treated with the vehicle FA. Kidney sections were stained for both CD45, a hematopoietic marker, and PDGFR-β, a mesenchymal marker, to identify bone marrow–derived fibroblasts. The number of $CD45^+$ and PDGFR-β^+ cells was significantly increased in the kidneys of WT mice after FA treatment. In contrast, the number of $CD45^+$ and PDGFR-β^+ cells was markedly reduced in the kidneys of IL-4Rα KO mice after FA treatment (Figure 1a and b). These data indicate that IL-4Rα plays an important role in the activation of bone marrow–derived fibroblast precursors in the kidneys after FA treatment.

IL-4Rα deficiency attenuates M2 macrophage polarization

The IL-4Rα signaling pathway plays an important role in M2 macrophage polarization.[24] We recently showed that myeloid fibroblasts are derived from monocytes through M2 macrophage polarization in a mouse model of renal fibrosis.[9] Therefore, we determined whether IL-4Rα regulates macrophage polarization and myeloid fibroblast formation in FA nephropathy. Kidney sections were stained for CD206, an M2 macrophage marker, and PDGFR-β. The number of $CD206^+$ and PDGFR-β^+ cells was substantially increased in the kidneys of WT mice after FA treatment. The number of $CD206^+$ and PDGFR-β^+ cells was significantly decreased in the kidneys of IL-4Rα KO mice after FA treatment (Figure 1c and d). These results indicate that bone marrow–derived fibroblasts originate from monocytes through M2 macrophage

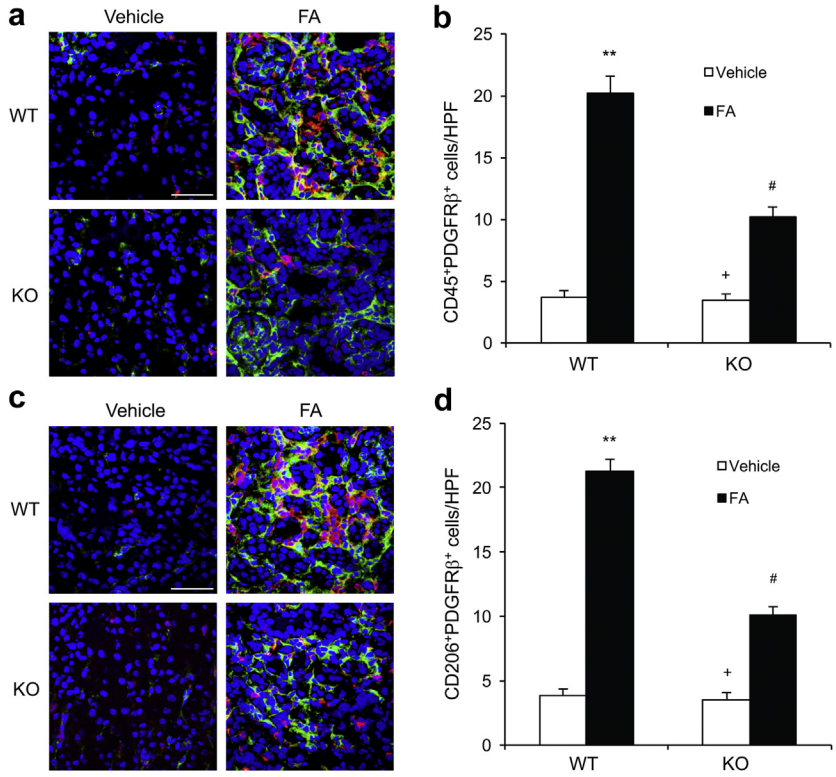

Figure 1 | Interleukin-4 receptor α (IL-4Rα) deficiency suppresses myeloid fibroblast accumulation and macrophage polarization in folic acid (FA) nephropathy. (**a**) Representative photomicrographs of kidney sections obtained from wild-type (WT) and IL-4Rα knockout (KO) mice at 2 weeks after vehicle or FA treatment, which were stained for a cluster of differentiation 45 (CD45; red), platelet-derived growth factor receptor-β (PDGFR-β; green), and 4′,6-diamidino-2-phenylindole (DAPI; blue). Bar = 50 μm. (**b**) Quantitative analysis of $CD45^+$ and PDGFR-β^+ fibroblasts in the kidneys. $^{**}P < 0.01$ versus WT-vehicle, $\#P < 0.05$ versus WT-FA, $+P < 0.05$ versus KO-FA; n = 6 in each group. (**c**) Representative photomicrographs of kidney sections stained for CD206 (red), PDGFR-β (green), and DAPI (blue). Bar = 50 μm. (**d**) Quantitative analysis of $CD206^+$ and PDGFR-β^+ fibroblasts in the kidneys. $^{**}P < 0.01$ versus WT-vehicle, $\#P < 0.05$ versus WT-FA, $+P < 0.05$ versus KO-FA; n = 6 in each group. HPF, high-power field. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.