Heterogenous Role of IRF4 in Kidney Fibrosis

A recent article by Sasaki *et al.*[1] entitled "Deletion of Myeloid Interferon Regulatory Factor 4 (IRF4) in Mouse Model Protects against Kidney Fibrosis after Ischemic Injury by Decreased Macrophage Recruitment and Activation" discussed the role of IRF4 in regulating macrophages during kidney fibrosis. They demonstrated that IRF4 expressed in myeloid cells could promote the development of tubulointerstitial fibrosis after ischemic reperfusion injury (IRI), possibly by orchestrating AKT-mediated monocyte recruitment to the injured kidney. However, Lassen *et al.*[2] demonstrated that IRF4 could suppress postischemic inflammation and prevent ARF, as evidenced by the aggravation of inflammatory cytokines and elevated serum creatinine and albumin in IRF4-deficient mice with IRI. Lorenz *et al.*[3] also delineated that IRF4 restricted CKD progression and kidney fibrosis after IRI, potentially by contributing to M2 macrophage polarization and inhibiting T helper 1 cell cytokine responses. Therefore, we have to wonder what is IRF4's role in kidney fibrosis after IRI?

The authors offered one hypothesis to explain this paradox: IRF4 expressed in different cell types may have different functions in response to acute injury, because IRF4 is also expressed in lymphocytes, including T and B lymphocytes, and IRF4 in T helper 17 cells could regulate anti-inflammatory IL-10 transcription. The authors did not seem to consider that there are far more infiltrating myeloid cells than infiltrating lymphocytes. Thus, the role of IRF4 in kidney fibrosis after IRI might depend mainly on its expression in myeloid cells. IRF4 has also been reported to negatively regulate the production of proinflammatory cytokines, including TNF-α and IL-6, by macrophages in response to Toll-like receptor stimulation, indicating IRF4 might promote macrophage activation.[4]

According to these published data, we hypothesize that IRF4 is capable of promoting migration, but also of inhibiting activation of macrophages. Macrophages in MΦ IRF4$^{-/-}$ mice could not migrate from bone marrow to peripheral blood, or from peripheral blood to injured kidneys. But the kidney-resident macrophages might undergo increased activation in MΦ IRF4$^{-/-}$ mice. The protection from kidney fibrosis in MΦ IRF4$^{-/-}$ mice may have resulted mainly from less infiltration of macrophages into injured kidneys. However, in IRF4 germline knockout mice, the inflammatory cells, including myeloid cells and lymphocytes, that reside in the kidney might be secreting more proinflammatory cytokines, such as TNF-α and IL-6, which partially contributes to an increased migration of macrophages. Thus, we suggest that the authors should detect the quantity of macrophages in bone marrow and peripheral blood in MΦ IRF4$^{-/-}$ mice, and conduct experiments to study the functions of resident macrophages in kidneys after IRI. The authors have illustrated that IRF4 could promote macrophage migration, possibly by activating phosphatidylinositol 3-kinase/AKT signaling. Moreover, searching concrete binding targets and direct downstream molecules of IRF4 could give more solid evidence of IRF4 positively regulating the migration of macrophages. Studying the molecular mechanism of IRF4 in kidney fibrosis after IRI might result in new strategies to treat this refractory kidney disease.

DISCLOSURES

All authors have nothing to disclose.

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Authors' Reply

We appreciate Liu and Zhang's interest in our recent study. We agree that, given previous studies by Lassen *et al.*[1] and Lorenz *et al.*,[2] our findings that selective myeloid deletion of interferon regulatory factor 4 (IRF4) decreased development of tubulointerstitial fibrosis[3] after ischemic kidney injury were somewhat unexpected. However, there are two crucial differences between our study and the two previous studies. Whereas we only deleted IRF4 expression in myeloid cells, both Lassen *et al.*[1] and Lorenz *et al.*[2] used mice with global IRF4 deletion, and IRF4 is also expressed in cells of nonmyeloid lineage.[4] In addition, we used a model of moderate kidney ischemia, whereas the ischemic injury in the previous studies was more severe. Therefore, we agree with Liu and Zhang that global IRF4 deletion and a more proinflammatory milieu may overcome the migratory defect in IRF4−/− myeloid cells and lead to persistent renal macrophage activation and subsequent fibrosis. Whether or not myeloid IRF4 deletion is deleterious in other models of CKD is an area of ongoing study in our laboratory.

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Need for a Validation Study before Using the Two-Step Algorithm for dd-cfDNA to Screen for Acute Rejection

The study by Bunnapradist *et al.*[1] proposes using a two-step algorithm threshold for donor derived cell free DNA (dd-cfDNA) to increase sensitivity for detection of acute rejection. Although this hypothesis is both tenable and biologically plausible, we have concerns if this study allows for any rigorously derived conclusions. Of the 41 patients in the study, 16 had (for cause) biopsies and 9 had biopsy-proven rejections. The new algorithm detected all nine acute rejections. Even though pre-ordained separate cutoffs were utilized, this is the first study to test this algorithm and thus must be considered as discovery and merely the first of many steps in biomarker assessment and ultimately utilization.[2] In addition, the improved test performance was accompanied by large confidence intervals and thus has a high risk of type 1 error due to the small sample size.[3] Given that many of the rejections were severe, it is unclear if this algorithm would retain this performance in the general transplant population.

The main challenge now will be conducting an adequately powered validation study upholding these results.[2] This can be difficult given the low prevalence of acute

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