IRF8-Dependent Type I Conventional Dendritic Cells (cDC1s) Control Post-Ishemic Inflammation and Mildly Protect Against Post-Ishemic AKI and Disease

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Background: Post-ischemic acute kidney injury and disease (AKI/AKD) involve acute tubular necrosis and irreversible nephron loss. Mononuclear phagocytes including conventional dendritic cells (cDCs) are present during different phases of injury and repair, but the functional contribution of this subset remains controversial. Transcription factor interworm regulatory factor 8 (IRF8) is required for the development of type 1 conventional dendritic cells (cDC1s) lineage. Several studies helped to define distinct cDC1 subsets according to the expression patterns of CD11b and CD11c in healthy kidney and lymphoid organs, of which IRF8 was significantly expressed in the CD11b+CD11c+ subset that mainly comprised cDC1s. Next, we applied a Irf8-deficient mouse line (Irf8f/f) to specifically target Clec9a-expressing cDC1s in vivo. During post-ischemic AKI/AKD, these mice lacked-cDCs in the kidney without affecting cDC2s. The absence of cDC1s mildly aggravated the loss of live primary tubule and decline of kidney function, which was associated with decreased anti-inflammation Tregs-related immune responses, but increased T helper type 1 (T1) regulatory and pro-inflammatory cytokines, infiltrating CD8+ T lymphocytes and acute tubule cell death, while we also observed a reduced number of cytotoxic CD8+ T cells in the kidney when cDC1s were absent.

Conclusions: Together, our data show that IRF8 is indispensable for kidney cDC1s. Kidney cDC1s mildly protect against post-ischemic AKI/AKD, probably via suppressing tissue inflammation and damage, which implies an immunoregulatory role for cDC1s.

PO0396 Myeloid Heparin-Binding Epidermal Growth Factor-Like Growth Factor (HB-EGF) Protects Against Ischemic AKI

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Background: Epidermal growth factor receptor (EGFR) activation plays an important role to mediate recovery of epithelial integrity following ischemic acute kidney injury (AKI) and subsequent development of interstitial fibrosis when recovery is incomplete. EGFR can be activated by a family of ligands. The ligand responsible for EGFR activation after AKI has not been previously identified. In response to various stimuli, EGFR can be translocated by its ligand HB-EGF. The present study examined the potential role of myeloid HB-EGF in recovery from ischemic AKI and subsequent development of fibrosis.

Methods: Wild type (HB-EGF+/+) or LysM-Cre; HB-EGFf/f (myeloid HB-EGFf/f) mice (male, 8 weeks old, C57BL/6J background) were uninephrectomized, immediately followed by unilateral ischemia-reperfusion with renal pedicle clamping for 31.5 min. Mice were sacrificed at different time points after ischemic AKI. Renal myeloid cells were isolated with a mixture of CD11b and CD11c microbeads. HB-EGFf/f mice had delayed functional recovery after ischemic AKI. At 28 days after AKI, myeloid HB-EGFf/f mice had more severe persistent kidney damage, indicated by higher KIM-1 mRNA and protein levels. Myeloid HB-EGF+/+ mice also had more renal immune cell infiltration, including macrophages, neutrophils, and lymphocytes. The myeloid HB-EGF+/+ mice exhibited more renal fibrosis, as indicated by quantitative Sirius red and Masson’s Trichrome staining and increased mRNA and protein levels of profibrotic and fibrotic components more renal fibrosis, as indicated by quantitative Sirius red and Masson’s Trichrome staining and increased mRNA and protein levels of profibrotic and fibrotic components. Downregulated genes included 33, Bmp6, Ccl12, and Fzd1. Regulated genes included Clec4a, Ifih1. Gene ontology analysis determined significant enrichment in the regulation of Wnt signaling and innate immune response activating signal transduction.

Conclusions: Myeloid HB-EGF activation leads to profibrotic programming of the myeloblast and interstitial fibrosis. Future studies will determine the specific epigenetic pathways that may be significantly changed by HDAC1 activation leading to maladaptive interstitial fibrosis.

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PO0399 Ischemia Reperfusion Activation of Kidney HDAC1 Results in Interstitial Fibrosis

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Background: Following a kidney ischemic event the chromatin remodeling enzyme, histone deacetylase-1 (HDAC1), is activated in many cell types of the kidney including fibroblasts/pericytes. Pharmacological inhibition of HDACs can attenuate ischemia-reperfusion-injury (IRI) mediated interstitial fibrosis. In this study, we tested the hypothesis that fibroblast/pericyte HDAC1 activation promotes interstitial fibrosis.

Methods: Tamoxifen inducible, fibroblast/pericyte HDAC1 knockout (KO) mice (HDAC1fl/fl, Col2a1-CreER) and littermate controls (HDAC1f/f) were used. Male and female mice (8-10 wks of age) were given tamoxifen i.p. and IRI or sham surgery was performed after a 2-week tamoxifen washout period. A mild, 18 min, bilateral, warm IRI was used, and samples collected over 4 weeks. Additional groups of mice underwent unilateral ureteral obstruction (UUO) for 48 h. In vitro experiments with kidney fibroblasts cells (NRK49F) overexpressing HDAC1 KO mice (NRK49F+HDAC1) were used for RNA-sequencing studies.

Results: HDAC1 KO was confirmed in myofibroblast cells by co-immunolocalization of HDAC1 and platelet-derived growth factor receptor beta or a-smooth muscle actin (α-sm) in the kidneys of IRI mice. 24 h post ischemia there was a tripling of plasma creatinine (Pcr) in all IRI mice, regardless of sex or genotype. 2- and 4-weeks after IRI, Pcr were similar to sham values for all mice. However, the male control IRI mice had significant interstitial fibrosis but this was attenuated in the KO male IRI mice. The female mice, regardless of genotype, had very mild kidney damage and interstitial fibrosis at 4 weeks. 2- and 4-weeks after IRI, Pcr were similar to sham values for all mice. However, the male control IRI mice had significant interstitial fibrosis but this was attenuated in the KO male IRI mice. The female mice, regardless of genotype, had very mild kidney damage and interstitial fibrosis at 4 weeks. 2- and 4-weeks after IRI, Pcr were similar to sham values for all mice. However, the male control IRI mice had significant interstitial fibrosis but this was attenuated in the KO male IRI mice. The female mice, regardless of genotype, had very mild kidney damage and interstitial fibrosis at 4 weeks. 2- and 4-weeks after IRI, Pcr were similar to sham values for all mice. However, the male control IRI mice had significant interstitial fibrosis but this was attenuated in the KO male IRI mice. The female mice, regardless of genotype, had very mild kidney damage and interstitial fibrosis at 4 weeks.