

**Myeloid Cell HO-ming in AKI**

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Heme oxygenase 1 (HO-1) is a readily inducible enzyme that converts highly reactive free heme molecules into carbon monoxide, iron, and biliverdin. Through this action, HO-1 influences reactive intermediate production, modulates the immune system, and affects cell survival in multiple pathogenic situations. In humans, HO-1 deficiency is associated with severe hemolysis, dysregulated inflammation, renal abnormalities, and early death. After stress or pharmacologic manipulation, HO-1 is upregulated in numerous tissues including the renal vasculature, tubular epithelial cells, and renal interstitial...
cells expressing the resident macrophage/dendritic cell (DC) marker CD68. Novel techniques and mouse models have recently begun to reveal how HO-1 in different cell types influences disease. For example, use of a tissue-specific knockout strategy demonstrated that HO-1 expression in myeloid cells (including neutrophils, monocytes/macrophages, and DCs) is critical for limiting pathology in a mouse model of multiple sclerosis, because myeloid-specific HO-1 deficiency resulted in chronic activation of DCs and enhanced accumulation of proinflammatory T cells in the spinal cord. Alternatively, HO-1 deletion exclusively from hepatocytes or myeloid cells improves ischemia does not result in significant renal injury or dysfunction or any mortality in wild-type control mice. The clinical relevance of HO-1 in AKI was recently bolstered by several important studies, including the demonstration that HO-1 levels in plasma and urine reflect intrarenal HO-1 activity and increase specifically in human AKI. Furthermore, transgenic expression of human HO-1 in HO-1−/− mice reverses the heightened sensitivity to rhodomyelosis and cisplatin nephrotoxicity. The proinflammatory immune response observed in AKI models is well known to enhance the direct nephrotoxic or ischemic damage to renal cells, and HO-1 has direct effects on numerous types of immune cells. Of note, infusion of macrophages that overexpress HO-1 after IRI ameliorates kidney dysfunction in mice, and global knockout of HO-1 promotes renal inflammation in AKI models. In this issue of JASN, Hull et al. significantly advance our understanding of the role of HO-1 that is expressed in myeloid immune cells in the kidney IRI model.

Using flow cytometry to carefully examine the effect of global HO-1 deficiency on renal inflammation during reperfusion, a marked increase in the number of neutrophils and monocytes/macrophages infiltrating the kidney was observed at 1 day of reperfusion in HO-1 knockout (KO) mice versus controls. This finding is in line with the renal-protective and anti-inflammatory role attributed to HO-1 in IRI. Strikingly, at the same time, there was a dramatic reduction in the number and proportion of renal DCs after ischemia in the HO-1 KO mice. DCs are known to traffic from the posts ischemic kidney to the renal draining lymph node where they present antigens to T cells, and enhanced trafficking could explain the reduction in renal DCs observed in HO-1−/− mice. DCs are key modulators of different types of AKI, which can either protect the kidney from injury, or promote renal injury, depending on the type of injury and the type of activation signals received by the DCs, so enhanced understanding of DC activation and trafficking in AKI may lead to new therapeutic approaches. To elegantly address the question of whether HO-1 influences the emigration of renal DCs to lymphatic organs, syngeneic kidney transplants were performed implanting HO-1−/− or HO-1+/− kidneys from mice that ubiquitously express green fluorescent protein (GFP) into GFP-negative recipients. In these experiments, the warm ischemic time for each graft was tightly controlled and limited to 25 minutes. Using immunofluorescence microscopy and flow cytometry, the number of HO-1+/+ and HO-1−/− DCs was quantified in the spleen, renal draining lymph node, and mesenteric lymph node. This experiment clearly demonstrated that HO-1+/+ MHC-II+ DCs traffic in greater numbers to each of these extrarenal lymphoid organs as early as 1 day after transplantation. To complement the transplantation study, mice lacking HO-1 expression solely in myeloid immune cells and control mice were subjected to 25 minutes of bilateral renal IRI. After 3 days of reperfusion, analysis of renal immune cells by flow cytometry demonstrated that lack of HO-1 expression in myeloid cells alone was sufficient to cause the reduction in renal DCs and increase in infiltration of monocytes/macrophages; however, myeloid-specific deletion of HO-1 did not result in the enhanced renal neutrophil accumulation observed in the global HO-1−/− mice after IRI. Thus, expression of HO-1 by renal DCs appears to promote their retention in the postischemic kidney and HO-1 expression in monocytes/macrophages impairs their ability to accumulate in the kidney after ischemia.

To address the biologic significance of the altered immune cell trafficking in the myeloid-specific HO-1−/− mice, renal function, histology, and markers of fibrosis were monitored for 7 days after ischemia. No difference in initial renal injury or dysfunction was noted between controls and myeloid HO-1−/− mice. However, at 7 days after IRI, enhanced renal function was observed in control mice and more fibronectin and collagen expression was detected in the kidneys of HO-1−/− mice. The reasons for impaired renal recovery and increased expression of markers of fibrosis have not been determined, but could be due to several possibilities. First, HO-1 is preferentially expressed in M2 macrophages and associated with their function, and the in situ switch of proinflammatory M1-type macrophages to M2 in the kidney after IRI is required for appropriate renal recovery. Second, retention of HO-1−/− expressing DCs in the kidney may promote the function of kidney-infiltrating regulatory T cells that positively influence the recovery of renal function and reduce fibrosis after IRI, because expression of HO-1 in DCs is required for optimal regulatory T cell function. Another possibility is that the increased trafficking of DCs from the ischemic kidney to secondary lymphoid organs may induce a more robust adaptive immune response to the injured kidney, limiting the ability of...
the kidney to recover. None of these possibilities are mutually exclusive and there are numerous other possibilities that may explain why the lack of HO-1 in myeloid cells inhibits recovery from AKI.

Another interesting finding presented in the article by Hull et al.\(^9\) is that although global HO-1 deficiency caused enhanced neutrophil homing to the kidney after IRI, myeloid-restricted HO-1 deficiency did not cause increased neutrophil accumulation.\(^9\) This is in contrast with the parallel effects of either global or myeloid-specific deletion of HO-1 on monocyte/macrophage infiltration to and DC emigration from the injured kidney, illustrating the complexity and specificity of the effects of HO-1 expressed in different cell types in kidney IRI.

Given the human relevance of HO-1 in AKI and the growing understanding of the myeloid cells in renal health and disease,\(^{26}\) these studies by Hull et al.\(^9\) provide the foundation for a new area of AKI research.

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DISCLOSURES

None.

REFERENCES


See related article, “Heme Oxygenase-1 Regulates Myeloid Cell Trafficking in AKI,” on pages 2139–2151.