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CD169+ Macrophages: Regulators of Neutrophil Trafficking to Injured Kidneys

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Ischemia-reperfusion injury (IRI) is a major cause of AKI. Despite increasing knowledge of the multifactorial pathophysiology of IRI, which includes epithelial and endothelial cell injury, inflammatory cell responses, and generation of soluble mediators, there is no effective treatment except for supportive care. Inflammation is well established as a driving force that initiates injury and is increasingly implicated in the long-term

sequelae of AKI.[1] The inflammatory response during IRI includes proinflammatory cytokine release, endothelial cell activation, and tissue infiltration by neutrophils, macrophages, and lymphocytes.[1] Several reports demonstrated that depletion or blockage of leukocytes ameliorates the inflammatory response but can also be detrimental in some cases.[1] Although monocytes/macrophages and neutrophils are known to be critical effectors of the inflammatory response, further dissection of their early molecular events during AKI is critical for clarifying their roles and developing novel therapeutics. In this issue of *JASN*, Karasawa *et al.* have identified a subset of peripheral blood monocytes and kidney-resident F4/80+ macrophages, categorized as CX3CR1hiLy6CloCD169+ cells, that suppress the excessive activation of endothelial cells and neutrophil infiltration in IRI kidneys.[2]

Neutrophil infiltration occurs in ischemic kidneys within the early few hours of reperfusion, which has primarily been demonstrated in experimental models.[3] Neutrophils exacerbate ischemic injury through blockage of capillaries (no-reflow phenomenon), release of reactive oxygen species and inflammatory cytokines including IFN-γ and IL-17, and disrupting the endothelial and epithelial barriers during their transmigration into the interstitial compartment.[4] Depletion of neutrophils, as well as blocking neutrophil transmigration to the kidney, can improve tissue damage after IRI, although some studies have failed to demonstrate a protective effect.[3]

Recruitment and transendothelial migration of neutrophils in capillary venules is a key event in the inflammatory response against tissue damage. Although the precise regulation of this process is incompletely understood, a cascade of interactions occurring between blood-borne neutrophils and the endothelium (characterized by rolling, firm adhesion, and extravasation) is required for neutrophil recruitment to the inflammation site. Resident dendritic cells play an important role in neutrophil recruitment by generating a potent chemotactic gradient with the release of TNF-α, IL-6, monocyte chemotactic protein 1, regulated on activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein 2, and keratinocyte-derived chemokine (the mouse analog of human IL-8).[3] IL-8 plays a crucial role in neutrophil recruitment and mediates tissue injury *via* cytokines, free radical intermediates, and proteases.[1]

Another key event in neutrophil recruitment is endothelial cell activation, which leads to an increase in vascular permeability and upregulation of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and P-Selectin.[1,3] These molecules facilitate neutrophil adhesion to endothelial cells and their extravasation, especially in the outer medullary region.[3] A key role for ICAM-1 in IRI-induced inflammation has been established by a number of studies using mice deficient for ICAM-1 or blockade of ICAM-1 in wild-type (WT) animals.[1] A role for tissue-resident macrophages and for the perivascular subset of macrophages in neutrophil recruitment was recently implicated in the induction of tissue inflammation.[5]

Monocyte/macrophage lineage expresses several surface markers, including CD11b, CD11c, F4/80, Ly6C, and CX3CR1.[6] These markers can be simultaneously expressed in both pro- and anti-inflammatory monocytes/macrophages and/or bone marrow–derived and kidney-resident monocytes/macrophages in specific disease states. The two distinct subsets of peripheral monocytes differ in their expression levels of CX3CR1 and Ly6C.[7] $CX3CR1^{hi}Ly6C^{lo}$ monocytes patrol the surface of endothelial cells to maintain endothelial integrity, whereas the $CX3CR1^{lo}Ly6C^{hi}$ monocytes are recruited selectively to the inflamed tissue where they differentiate into macrophages and dendritic cells.[8,9]

CD169, sialic acid–binding IgG-like lectin (Siglec)-1, originally referred to as a lectin-like receptor, is a unique cell surface receptor of macrophages.[10] $CD169^{+}$ macrophages during health are mainly present in secondary lymphoid organs, such as the lymph node and spleen. However, during disease, they are found in many other organs and can serve as a regulator of adaptive immunity through the activation of T and B cells, as well as antigen presentation.[11] $CD169^{+}$ macrophages have been associated with the progression of inflammation-related diseases, including arthritis and GN,[11] and the precise mechanisms remain undefined.

Although the presence of $CD169^{+}$ macrophages, referred to as $Siglec-1^{+}$ macrophages, was previously reported,[12] their role in kidney diseases is relatively unknown. In this issue of *JASN*, Karasawa *et al.* report the novel finding that $CD169^{+}$ macrophages, derived from $CX3CR1^{+}$ monocytes and referred to as $CX3CR1^{hi}Ly6C^{lo}CD169^{+}$ cells, protect the kidney from ischemic AKI.[2] Compared with controls, depletion of the $CD169^{+}$ macrophages by diphtheria toxin resulted in more severe IRI-induced tubular necrosis and functional impairment, as well as higher mortality. To define the origin of the $CD169^{+}$ macrophage, an elegant parabiosis model (surgically joining together two organisms which leads to shared circulatory system) was established using CD169-cre-YFP and ROSA26-YFP mice, which demonstrated that a significant portion of the $CD169^{+}$ macrophages reside in the kidney and only a minor portion were derived from the circulating monocyte population. In addition, Karasawa *et al.* found that kidney-resident $CD169^{+}$ macrophages reside along the vasculature, similar to pericytes, suggesting a role for maintaining the vascular integrity.[2] IRI results in enhanced vascular permeability due to the disintegration of the junctional barrier between endothelial cells, which contributes to the recruitment of leukocytes into the injured kidney. Indeed, deletion of the $CD169^{+}$ monocytes/macrophages exacerbated recruitment of neutrophils into the kidney after IRI. The primary mechanism for the increased neutrophil recruitment into injured tissue was due to elevated expression of endothelial ICAM-1, as well as an increase of chemokine (C-X-C motif) ligands CXCL1 and CXCL2. Blockage of ICAM-1 using an ICAM-1–specific antibody prevented IRI-induced lethality in mice, confirming its important role. *In vitro* experiments were conducted to determine whether $CD169^{+}$ monocytes/macrophages may require direct interaction with endothelial cells to regulate ICAM-1 expression. During *in vitro* coculture experiments with leukocytes and endothelial cells seeded together, but not in transwell cultures, leukocytes from CD169-deleted mice markedly increased the expression of ICAM-1. This demonstrated the requirement for a direct interaction of leukocytes and endothelial cells in ICAM-1 upregulation.

To further elucidate details of this protective leukocyte population during AKI, total PBMCs were transferred into CD169-deleted mice before IRI. The survival rate of CD169-DTR mice from lethal AKI improved by 60%, whereas the injection of WT $Ly6G^{hi}$ neutrophils did not improve the survival rate.[2] Furthermore, transfer of the fractionated $Ly6C^{lo}$ monocyte subtype into CD169-deleted mice improved the survival rate of the mice from kidney IRI compared with the same mice without transfer. Unexpectedly, $Ly6C^{hi}$ monocytes, considered an inflammatory monocyte, also showed mild protection from IRI. It is suggested that the protective effect of $Ly6C^{hi}$ monocytes may be due to switching to $Ly6C^{lo}$ monocytes, although further study is warranted to confirm this postulate. The monocyte transfer was associated with the suppression of ICAM-1, further confirming suppression of endothelial cell activation by subtype-specific monocytes/macrophages in kidney IRI. These data concur with a recently reported study in which perivascular macrophages directly affect the adhesive and transmigratory activity of blood neutrophils.[13]

Nonetheless, important issues remain to be addressed, including the question of whether $CD169^{+}$ monocyte transfer would be protective in the setting of IRI in WT mice, which could pave the way to translate the findings to humans. Recent reports showed that some monocyte/macrophage subsets, such as $Ly6C^{lo}$, are required for the prevention and recovery from acute injury in several tissues, including the kidney.[14,15] The kidney function of WT mice recovered at 48 hours post-IRI, whereas that of $CD169^{+}$ macrophage-depleted mice deteriorated.[2] The functional impairment observed at 48 hours after IRI by $CD169^{+}$ macrophage depletion could be associated with the impairment of repair process, as well as early injury. This possibility is further supported by the observation that after unilateral IRI in $CD169^{+}$ macrophage-depleted mice, tubular regeneration was impaired after 7 days, suggesting a possible role for $CD169^{+}$ macrophages in recovery after IRI. It should also be mentioned that renal resident and/or infiltrating monocytes, macrophages, and dendritic cells have overlapping phenotypes and surface marker expression. Because the $CD169^{+}$ macrophage was positive for Ly6C, a marker of dendritic cells, investigating the role of $CD169^{+}Ly6C^{+}$ dendritic cells would also be of interest in AKI.

In summary, the elegant mechanistic findings of Karasawa *et al.* demonstrate the role of the $CD169^{+}$ macrophage as a regulator of neutrophil recruitment and vascular homeostasis in ischemic kidneys.[2] Better understanding of the $CD169^{+}$ monocyte/macrophage functions, tissue factors that may trigger their activation, and the macrophage effectors that may

activate endothelial cells may provide novel treatment strategies for ischemic AKI.

DISCLOSURES

None.

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Understanding the Role of Rituximab in ANCA GN: Regressing toward the Mean

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Who in their right mind could argue? It made complete pathogenetic sense that remission induction could be accomplished by rituximab-induced depletion of circulating peripheral CD20-positive B cells in patients with ANCA vasculitis. ANCAs are bioindicators of inflammatory autoimmune conditions affecting small vessels, namely granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic GPA. Since the initial discovery of ANCAs directed against myeloperoxidase (MPO) and/or proteinase 3, there has been an explosion of insight into pathogenetic disease mechanisms that has directly led to novel therapeutic inroads.[1] For example, ANCAs bind to and activate circulating neutrophils, thereby resulting in vascular inflammation, and the murine model of MPO-ANCA vasculitis revealed that ANCAs are necessary and sufficient to cause and perpetuate disease.[2,3] As with the use of plasma exchange to physically remove offending ANCAs, this compendium of data made a very strong case from a pathogenetic standpoint that the use of rituximab to deplete peripheral B cells would be beneficial. Furthermore, maintenance of this effect could be long lasting. In other words, ridding the inflammatory milieu of the etiologic stimulant could melt disease. It should be noted that this rituximab-induced melting is not instantaneous—it takes time. Rituximab is unable to deplete plasmablasts and plasma cells, because these cells do not harbor CD20 on their cell surface and are known to reside outside of the circulation; thus, ANCA titers fall slowly over time in individuals treated with rituximab.[4–6] Unfortunately, kidney involvement is exceedingly common in GPA and MPA and typified by pauci-immune, necrotizing, and crescentic GN. The degree and duration of kidney involvement before treatment portend a worse prognosis, suggesting that glomeruli suffer until ANCAs are removed for good.[7]

It came as no surprise that both patients with ANCA vasculitis and their health care providers were enthusiastic about