various miR, and miR-21 is one of the most intensively studied miR regulated by HIF-1.[11] The complex network of HIF-1 and miR is a critical subject for future studies.

PTEN is also known to be involved in a network of interactions with p53, which transcriptionally activates genes involved in cell cycle control and apoptosis. PTEN regulates p53 protein levels and transcriptional activity through both phosphatase dependent and independent mechanisms. A crucial role of p53 in tubules damaged by ischemia reperfusion has been reported by the authors' group and others,[12–14] and a role of p53 in the HIF-1/miR-687/PTEN signaling pathway is an important issue to be pursued.

Finally, hypoxia is a condition of energy depletion caused by disturbance of aerobic respiration of mitochondria. HIF-1 plays a critical role in energy metabolism, reprogramming cellular metabolism toward enhanced glycolysis and reduced oxidative phosphorylation (*i.e.*, Warburg effect). Recent studies clarified the functional role of HIF-1-induced large intergenic ncRNA-p21 (lincRNA-p21) in this metabolic reprogramming. LincRNA-p21 is rapidly induced by HIF-1α, and lincRNA-p21 then disrupts the HIF-1α von Hippel–Lindau interaction, stabilizes HIF-1α, and increases expression of HIF-1α-responsive genes (*e.g.*, glycolytic enzymes Glut1 and LDHA), thereafter promoting glycolysis and reprogramming cellular metabolism.[15] Regulation of energy metabolism by the HIF-1/miR-687/PTEN signaling pathway should be investigated in the future.

This study is well-conducted with sophisticated techniques, validating the observations using independent methodologies from various aspects. Thomas Edison gave us the aphorism "Everything comes to him who hustles while he waits" that emphasizes the importance of the energetic and ongoing pursuit of a given goal. The work of Bhatt and colleagues is a wonderful example of how the vigorous and unremitting exploration of mechanisms underlying acute ischemic injury can lead to rewarding insights, including those, as shown in this study, regarding miR-687 and HIF delineation of the novel HIF-1/miR-687/PTEN signaling pathway providing new insights into the understanding of the pathogenesis of AKI to develop novel therapeutic approaches.

DISCLOSURES

None.

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See related article, "MicroRNA-687 Induced by Hypoxia-Inducible Factor-1 Targets Phosphatase and Tensin Homolog in Renal Ischemia-Reperfusion Injury," on pages 1588–1596.

The Janus Faces of IL-6 in GN

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In the current burgeoning biologic era of autoimmunity therapeutics, an expanding array of specific molecular targets are being used to dismantle the components of the dysregulated immune system felt to be responsible for tissue injury. We are learning that what works well for one disease may not work for another, that there may be consequences that may not have been predicted based on our current knowledge of the biology of the target in question, and that the promised reduction in infectious adverse events has been difficult to realize in practice. Intelligent use of novel biologic agents should be based on sound knowledge of both their *in vitro* and *in vivo* effects. In this issue of *JASN*, a study by Luig *et al.*[1] presents compelling data on the significance of IL-6 in modulating macrophage-mediated renal injury in GN.

IL-6 is a multifunctional cytokine regulating immune activation. It is released alongside other inflammatory effectors and plays a pivotal role in control of the acute phase response, stimulation of B cell differentiation into antibody-forming cells, and development of Th17 cells. Its inflammatory actions have made it a key therapeutic target for various chronic inflammatory conditions. Luig *et al.*[1] demonstrate upregulated macrophage infiltration into the glomeruli of mice treated with anti–IL-6 receptor (IL-6R) antibody or anti–IL-6 antibody, leading to unexpected worsening of nephrotoxic nephritis (NTN; a commonly used model of crescentic GN), despite a marked reduction in systemic cytokine and pathogenic antibody responses. In elegant experiments using mice lacking IL-6R on macrophages but not on adaptive immune cells, which were generated by intercrossing $LysM^{Cre}$ with $IL\text{-}6R\alpha^{flox/flox}$ mice, Luig *et al.*[1] provide strong evidence of a role for IL-6 in subduing the proinflammatory macrophage expansion in this condition.

The mechanistic story presented depends on the IL-6R being restricted to macrophages in inflamed kidney. However, this receptor is also expressed on neutrophils and basophils. Importantly, the elegant key experiments in the $LysM^{Cre}$ conditional knockouts would also cause loss of the IL-6R from neutrophils. An alternative hypothesis is thus conceivable, which suggests that IL-6 is acting through neutrophils (which are key pathogenic cells in NTN) to dampen the innate immune response. Arguing against this, however, is the fact that IL-6R expression is low on neutrophils, with only approximately 5% of cells being positive. In addition, although removing IL-6 from the system in the IL-6 null mice led to a marked increase in macrophage expansion after immunization, neutrophil recruitment was in fact reduced, indicating that IL-6 has divergent effects on neutrophils and macrophages. Thus, the anti-inflammatory effect of IL-6 in experimental GN does appear to be focused on potentially histotoxic macrophages, with any minor neutrophil effects being proinflammatory.

IL-6 is an important stimulator of Th17 cell expansion, for which there is evidence of a pathogenic role in crescentic GN.[2] Therefore, one would expect blockade of this cytokine to retard glomerular injury in a manner similar to the beneficial effect observed in rheumatoid arthritis.[3] The observation of the opposite effect highlights a potential pantheon of differing effects of IL-6 on inflammation, which must have important implications for its use in specific autoimmune and autoinflammatory diseases. To begin to understand this, one must have an appreciation of the ways in which IL-6 signals. IL-6 is a pleiotropic glycoprotein with a molecular mass of 26 kD. IL-6 was first identified as B cell differentiation factor or B cell stimulatory factor 2, which was found to be identical to hybridoma growth factor, to hepatocyte-stimulating factor with its promotion of synthesis of acute phase proteins, and to IFN-γ2. After binding to IL-6R (CD126), an 80-kD transmembrane protein, the complex consisting of IL-6 and transmembrane IL-6R associates with the signal-transducing molecule gp130, resulting in activation of Janus kinase–mediated downstream signaling pathways. These include phosphoinositol-3 kinase, signal transducer and activator of transcription 3 (STAT3), extracellular signal–regulated kinase, suppressor of cytokine signaling 3 (SOCS3), and mitogen-activated protein kinase governed pathways that lead ultimately to gene transcription. It is through STAT3 activation that IL-6 controls the key T cell switch from regulatory T cells to Th17 cells in the presence of TGF-β. Importantly, in the study by Luig *et al.*,[1] the renal Th17 response remained intact after IL-6 blockade.

However, transmembrane IL-6R expression is limited to cells such as hepatocytes and some leukocytes, whereas gp130 is expressed on a wide array of cells. Thus, in addition to this classic signaling pathway, a trans-signaling pathway exists whereby soluble IL-6R in blood, lacking the cytoplasmic region, binds to IL-6 and the complex binds independently to gp130, leading to signaling cascade activation. It has been suggested that this IL-6 trans-signaling largely mediates anti-inflammatory processes, whereas the classic signaling pathway promotes inflammation. However, in this study, blockade of trans-signaling using a sgp130Fc fusion protein had no effect, suggesting that, in this setting, classic IL-6 signaling was responsible for the brake on proinflammatory M1 macrophages.

With regard to kidney disease, IL-6 has been studied for a number of years, with diverse and seemingly contradictory effects noted. It is synthesized by leukocytes and intrinsic renal cells (including mesangial cells and podocytes) in response to proinflammatory signals such as IL-1, TNF, PDGF, damage-associated molecular patterns, and pathogen-associated molecular patterns, and leads to mesangial and epithelial cell proliferation.[4] Urinary levels are elevated in proliferative glomerulonephritides such as IgA nephropathy, mesangioproliferative GN, and lupus nephritis, in which elevated serum levels are also found and increased glomerular expression can be demonstrated,[5] both of which correlate with lupus disease activity. Unlike this study, in murine models of lupus nephritis, exogenous IL-6 accelerates disease and IL-6 blockade attenuates it,[6] with reduction in circulating autoantibodies, reduction in mesangial proliferation, and improvement in GFR. However, as with many

other cytokines, the proinflammatory and proliferative actions are countered by anti-inflammatory effects, as demonstrated using *in vitro* models and *in vivo* through the use of knockout animals. In glomerular disease, it was shown almost 20 years ago that infusion of IL-6 into a rodent anti–glomerular basement membrane model reduced proteinuria, glomerular thrombosis, and macrophage infiltration,[7] which is echoed in the study by Luig *et al.*[1] IL-6 can exert an anti-inflammatory response in monocytes and glomerular epithelial cells through termination of effects mediated by IL-1 and TNF. In models of endotoxemia, IL-6 exerts a protective role, reducing the production of TNF, macrophage inflammatory protein 2, IFN-γ, and GM-CSF, augmenting cortisol secretion, and reducing neutrophil influx into target organs.[8] The anti-inflammatory effect of IL-6 is mediated *via* the action of SOCS3, which can lead to inhibition of IFN-γ responses.[9] More recently, using an *in vitro* model, podocyte release of IL-6, again signaling *via* SOCS3, was shown to mediate an anti-inflammatory effect on microvascular glomerular endothelial cells (but not umbilical vascular endothelial cells), inhibiting TNF-mediated neutrophil recruitment and transmigration.[10] These *in vivo* and *in vitro* data demonstrate the complex duality of IL-6 action.

Therefore, what is the effect of targeting IL-6 in kidney disease? Is the overall effect to reduce or exacerbate renal inflammation?

Anti–IL-6 or IL-6R therapies have been developed for use in numerous immune-mediated diseases. To date, there have been limited trials targeting IL-6 in renal patients, which have focused primarily on lupus nephritis. Phase I studies have reported some reduction in autoantibodies and variable changes in renal parameters. There are additional anecdotal case reports of benefit from IL-6–targeted therapy in crescentic GN complicating rheumatoid vasculitis, in myeloperoxidase-ANCA–associated vasculitis, mesangial proliferative GN secondary to Castleman's disease, and nephrotic syndrome complicating AA amyloidosis, all arguing in favor of a predominantly proinflammatory effect of IL-6 in these conditions. By contrast, a single report of immune complex GN arising *de novo* in a patient with rheumatoid arthritis treated with tocilizumab[11] suggests that, like anti-TNF therapy, we need to be wary of the duality of IL-6 action and its potential for augmenting certain renal lesions.

Importantly, it should be remembered that the IL-6 role in inflammation extends well beyond the immune system and the kidney, with high levels of expression in atherosclerotic plaques and elevated serum levels being associated with increased coronary artery disease and morbidity from hypertension, left ventricular hypertrophy, and development of insulin resistance. Single nucleotide polymorphisms in the IL-6 promoter or IL-6R are associated with elevated levels of circulating IL-6, lower levels of C-reactive protein (mimicking the therapeutic effects of anti–IL-6R antibodies such as tocilizumab), and a significantly reduced odds ratio of coronary artery events,[12] which are common in renal patients with immune-mediated nephritis, such as lupus nephritis or ANCA-associated vasculitis. Thus, there could be additional overall benefits of IL-6 blockade in these patients, which would not be apparent in short-term studies using *in vitro* or *in vivo* experimental models.

Janus presided over the beginning and ending of conflict, and hence war and peace. IL-6 appears to play a similar role in both initiation and prevention of glomerular inflammation.

DISCLOSURES

None.

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