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See related article, "Mutations in *INF2* Are a Major Cause of Autosomal Dominant Focal Segmental Glomerulosclerosis.," on pages 239–245.

Macrophages in Kidney Repair and Regeneration

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Appreciation of a central role for recruited monocyte-derived macrophages to repair organs after injury is gaining considerable momentum. In the past 2 years, monocyte-derived tissue leukocytes have been identified as orchestrators of repair in skin, muscle, gut, brain, and heart. Macrophages, however, exhibit considerable plasticity in their phenotype and can polarize into functional states that additionally contribute to tissue injury or fibrosis. These functional states have become known as M1 for pro-injurious functions and M2 for wound-healing functions, but this nomenclature belies the complexity of macrophage diversity. The phenotypic switch that is central to macrophage-directed repair and the effectors of this repair merit further study.

In kidney diseases, monocyte-derived tissue effector cells, known as macrophages, have a bad reputation along with neutrophils as drivers of tissue injury and fibrosis.[1] Although this is certainly true and therapies that target injurious macrophages and injurious mechanisms of the innate immune system that wreak havoc inappropriately in our organs are greatly welcomed, it seems that proinflammatory macrophages and neutrophils are *the exception that proves the rule.*[1,2].

What is the rule? The innate immune system serves to police our organs and promote repair and regeneration without causing injury. Only in overwhelming circumstances such as infection or severe tissue injury do macrophages activate sterilizing and injurious programs. Macrophages are particularly adept at clearing debris, extracellular matrix, immune complexes, and dead cell products of tissue injury and most of the time perform such tasks silently.[3] The same sort of macrophages also seem to have the capacity to release almost every cytokine and growth factor described in the literature. Coordinated release of many of these factors promotes organized tissue regeneration including basement membrane synthesis, cell proliferation, cell migration, and dampening of the inflammatory response.

In some circles, reparative monocyte-derived cells with avidity for microvascular repair are known as endothelial progenitor cells (EPCs). EPCs are now widely described to promote capillary repair and restoration by a number of mechanisms, including canalization of new capillary tracts, temporary (days to weeks) replacement of endothelial cells in areas of denuded capillary basement membrane, new capillary basement membrane synthesis, cytokine release that promotes endothelial cell proliferation, and adoption of pericyte functions.[4,5]

But can the endogenous reparative functions of macrophages be harnessed for good in the kidney? It seems so. Lee *et al.*[2] in this issue of *JASN* set out to test whether deliberate manipulation of inflammatory monocyte/macrophages alters the course of injury and repair in kidney ischemia reperfusion injury (IRI). In loss-of-function studies, the authors specifically ablate monocytes and macrophages using a toxic drug encapsulated within liposomes. Ablation at the onset of injury was protective, whereas ablation during the repair phase was deleterious. In gain-of-function studies, they adoptively transferred into the circulation macrophages that were recruited to the injured kidney. Macrophages primed with IFN-γ to adopt an M1 or injurious phenotype exacerbated injury, but adoptively transferred macrophages primed to exhibit a wound-healing phenotype lacked this capacity. Typical macrophage M1 markers such as nitric oxide synthase 2 were found in early kidney injury, whereas typical macrophage M2 markers such as the mannose receptor were detected during the repair phase. When the investigators adoptively transferred M1-primed macrophages to the kidney early after injury and then tracked them,

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they noted the transferred cells activated the M2 markers, the mannose receptor, and Arginase with time, indicative of a phenotypic switch.

M2-primed macrophages had a specific capacity to stimulate tubular cell proliferation *in vitro* and *in vivo*. Thus, during the normal repair of kidney injured by ischemia, macrophages acquire regenerative functions, which included stimulating successful epithelial proliferation.

These findings are entirely consistent with several other studies published in the past year in which macrophages or the monocyte-derived dendritic cells were ablated during the repair phase of IRI in the kidney with deleterious outcomes.[6–8] These other studies used a genetic system to ablate macrophages, which has significant methodologic differences compared with the report from Lee *et al.*[2] Nevertheless, the consistent message is that macrophages promote normal repair and regeneration after injury, and a major component of that process is stimulation of epithelial regeneration by cell–cell cross-talk.

In none of these studies or the study by Lee *et al.*,[2] however, has the role of macrophages in repair of the capillaries of regenerating kidney been studied, yet the parallels between monocyte-derived EPCs in microvascular repair and macrophages in epithelial repair are striking, and vital peritubular capillaries are severely disrupted in ischemic kidney injury.[9] In models of single toxic, ischemic, or surgical injury of heart, skeletal muscle, gut, pancreas, liver, brain, and skin, a wave of repairing monocyte-derived cells moves into the tissue and orchestrates a repair and regeneration program.[4,10–14] This suggests the observations in kidney repair reflect a generalized function of the macrophage, one that can be harnessed for therapeutic benefit.

What next? Although we now know that macrophages can promote repair, the studies of Lee *et al.*[2] tell us this occurs at the expense of M1 activation followed by a phenotypic switch and that M1-activated macrophages can be deleterious. With the advent of cell therapy, it may be possible to administer primed monocytes to the circulation that do not require M1 activation to deliver regenerative functions to the kidney. Studies of this nature (IL-10–expressing adoptively transferred macrophages) were performed nearly 10 years ago in the laboratory of David Kluth and Andy Rees in rat models of glomerulonephritis, but, although convincing, this line of investigation never made it to human studies.[15] Perhaps we should revisit this as a therapeutic strategy.

It is clear that while in the experimental tissue milieu of post-IRI kidney macrophages cause repair in rodents, this environment may not often be present in human disease, and many studies of rodents show that macrophages in chronic renal injury actually drive injury and fibrosis.[1] Understanding which tissue factors trigger a phenotypic switch toward tissue repair and regeneration and the macrophage effectors that bring about repair and regeneration may bear more therapeutic fruit for humans in need of macrophage-directed therapy in the future. To that end, several studies now implicate successful delivery of the macrophage cytokine IL-10 to the kidney or stimulation of macrophage Wnt signaling pathways in epithelium as potential therapeutic options for human disease.[6,16]

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DISCLOSURES

None.

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See related article, "Distinct Macrophage Phenotypes Contribute to Kidney Injury and Repair," on pages 317–326.

Identification of a Major Chronic Renal Failure Susceptibility Locus in Mice: Perhaps EGFR Determines What Happens to eGFR

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One of the most challenging issues facing modern renal science is finding at-risk genes for kidney disease, particularly those predisposing to the development or progression of chronic kidney disease (CKD). In human populations, the use of Genome Wide Association Screening (GWAS) has identified a number of loci associated with CKD.[1,2] Further refinements to GWAS techniques using admixture-mapping linkage disequilibrium also allowed for the identification of a locus on chromosome 22q12 that predisposes individuals of African descent to nondiabetic glomerular injury,[3,4] and recent analyses strongly indicate that the affected gene is *ApoL1*.[5,6] Undoubtedly, other genes are involved in development of progressive kidney disease.

Given the genetic complexity of essentially all modern human populations, investigators have also turned to model organisms to uncover mechanisms that predispose to renal progression. In this regard, the mouse provides distinct advantages[7] in that genetically well-characterized inbred strains abound and previous studies identified marked differences in susceptibility to the development of progressive kidney injury among strains.[8–11] In this issue of *JASN*, Laouari *et al.*[12] have taken advantage of this differential susceptibility and performed a genetic linkage analysis in mice to identify genes that predispose to renal progression after subtotal nephrectomy.

In previous studies, the authors identified the FVB/N mouse strain as susceptible to development of renal injury in response to either subtotal nephrectomy or continuous angiotensin II infusion.[13,14] In the current studies,[12] the authors phenotyped three other mouse strains and two F1 hybrids—C57BL/6, DBA/2, 129S2/Sv, (C57BL/6xDBA/2)F1 (B6D2F1), and (C57BL/6xSJL)F1—and found that all of these strains were resistant to development of injury within 8 weeks after ¾ nephrectomy, whereas FVB/N mice developed progressive worsening of renal function, proteinuria, and glomerular and tubulointerstitial damage. They then intercrossed FVB/N mice with B6D2F1 mice and found that 96% of male F1 offspring inherited the renal failure phenotype, whereas only 4% of females did. This gender-dependent phenotype was observed regardless of the direction of the crosses, arguing against gender-linked inheritance. To establish that this remarkable difference in sensitivity by gender was not the result of either X or Y chromosome transmission, they backcrossed F1 females to male FVB/N and B6D2F1 mice. The proportion of affected male and female offspring indicated autosomal transmission and suggested gender-specific penetrance, manifesting as a dominant trait in males and a recessive trait in females.

The authors then used these informative backcrosses to perform a GWAS using 64 microsatellite markers and identified a quantitative trait locus (QTL) on chromosome 6, called *Ckdp1*, which contains more than 400 genes. They were then able to refine the QTL further with additional markers, but the identified locus still spans a relatively large, gene-rich region. A total of 125,294 single-nucleotide polymorphisms were identified within the critical region, among which 9668 (7.7%) were polymorphic between FVB/N and both C57BL/6 and DBA/2. Of these 9668 single-nucleotide polymorphisms, 3904 are located within 104 previously annotated genes. It is noteworthy that this locus is syntenic with regions on human chromosome 2 and chromosome 3 that have also associated with CKD.[1,2,15,16]

The authors also previously reported an important role for the EGF receptor (EGFR) in mediating progressive renal injury.[17] EGFR can be activated by a family of growth factors in addition to EGF,[18] and in FVB/N mice, one of these EGF-like growth factors, TGF-α, increases in response to progressive renal injury, whereas blocking EGFR activation significantly decreases progressive kidney damage.[14,17] Of note, TGF-α is one of the genes that resides in the *Ckdp1* locus on chromosome 6.[12]

In further studies, Laouari *et al.* confirmed that renal expression of TGF-α increases in FVB/N mice after subtotal ne-

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