CHAPTER 29

Maladaptive Repair and Progression to CKD

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OBJECTIVES

This chapter will:

1. Describe the molecular and cellular events underlying acute kidney injury (AKI)–induced maladaptive repair.
2. Describe the possible role of different cell types in this process.
3. Define the pathogenic mechanisms of the transition from AKI to chronic kidney disease.

The outcome of acute kidney injury (AKI) may vary from complete resolution to an incomplete recovery of renal function, leading to chronic kidney disease (CKD) and subsequent progression towards end-stage renal disease (ESRD).[1] An increasing body of evidence, including observational studies and registry data, suggests a causal link between AKI and the consequent development of CKD.[2]

The outcome of AKI depends, at the tissue level, on the balance of adaptive and maladaptive repair.[3] Adaptive repair usually leads to resolution of renal injury without

long-term consequences. The hallmarks of such processes are a rapid recovery of renal function, resolution of inflammation, tubular proliferation, and decrease in damage biomarkers. Maladaptive repair, on the other hand, results in a stable reduction in kidney function associated with significant changes in renal architecture. The main features of this process are represented by the persistence of renal dysfunction, development of interstitial fibrosis, persistent expression of fibrogenic factors, and delayed resolution of inflammation. Maladaptive repair may occur in the tubular, vascular, and interstitial compartments in response to AKI, predisposing to the development of histologic changes featuring progressive renal injury.[3] Several animal models have been used to investigate the pathophysiology events of AKI-induced CKD.[3] Most models have used ischemia/reperfusion and in a minority of reports nephrotoxins and ureteral obstruction as primary injuries. Most of the models were in rats and mice, whereas only few studies were performed in larger animals such as pigs. In the majority of mice and in all of swine models, histologic evidence of fibrosis was the primary end point.

These animal models allowed us to realize that in AKI several processes are primed by injury and subsequent regeneration and involve resident and infiltrating cells. Tubular and endothelial cells always have been considered as the main actors in the pathogenesis of AKI and in the events leading to its resolution. However, in the last two decades we recognized the key role of other resident cells, including pericytes, in this complex scenario. Most of the research on the role of resident cells in the pathogenesis of maladaptive repair was focused on the attempt to define whether tubular cells, endothelial cells, or pericytes give origin to interstitial myofibroblast. Indeed the appearance of these cells within the interstitial space is the hallmark of maladaptive repair because these activated fibroblasts are responsible for the deposition of collagen and other components of extracellular matrix leading to interstitial fibrosis. On the other hand, the activation of the innate immune system always has been considered essential in the development of renal injury an in its repair, although evidence now supports a key role also for infiltrating B and T cells. All of these actors play a significant role in the maladaptive repair leading to the progression of AKI towards CKD.[3]

RESIDENT CELLS

Tubular Cells

Tubular cells always have been considered central in the development of AKI, although for a long period of time only as a target of injury.[4] In the last decades we realized that these cells play an active role in the pathogenesis of AKI. After a long-lasting discussion, their pivotal function in the repair process also is evident.[4] In this setting there is now substantial evidence that they are significantly involved in the maladaptive repair leading to CKD.[4] Indeed a significant amount of evidence suggests the involvement of tubular cells in the development of interstitial fibrosis, the hallmark of maladaptive repair. In this scenario, much of the attention has been focused for a long period of time on the ability of tubular epithelial cells to undergo epithelial to mesenchymal transition (EMT), whereby epithelial cells, under particular conditions, may transdifferentiate into myofibroblasts, the cells considered the main responsible of extracellular matrix deposition.[5] Recently, the role of EMT in the maladaptive repair of AKI has been doubted by several elegant studies that could not demonstrate any transdifferentiation of tubular cells in this setting.[6,7] Once EMT was excluded, the interest moved to the paracrine effects of tubular cells, potentially contributing to interstitial recruitment of inflammatory cells and interstitial fibroblasts and/or perivascular pericytes activation.[8] In normal conditions, tubular epithelial cells divide at a very low rate to preserve the physiologic turnover. In the repair phase of AKI, the number of proliferating cells increases exponentially to replace necrotic/apoptotic cells.[4] After mild injury, surviving tubular cells dedifferentiate transiently, proliferate to restore the cell number,[9] and then redifferentiate into specialized tubular cells.[10] Several observations suggest that the regulation of cell cycle after AKI may play a pivotal role in the pathogenesis of maladaptive repair. Indeed, in response to different type of stress, proliferating tubular cells may be arrested in the G2/M phase of the cell cycle.[11] This stop in cell division leads to an increased expression and release of profibrotic factors, including connective tissue growth factor (CTGF) and transforming growth factor-beta (TGF-β).[11] After AKI, the number of cells undergoing G2/M arrest directly and significantly correlates with the extent of fibrosis.[11] In addition, pharmacologic interventions that increase the number of tubular cells arrested in G2/M after AKI worsens kidney fibrosis, whereas interventions that enhance the movement through G2/M are associated with less fibrosis.[11]

The G2/M phase arrest may be, at least in part, the result of DNA damage associated with severe tubular injury.[11] The presence of DNA damage associated with G2/M arrest and secretion of fibrogenic mediators has been reported in mice models but also may be observed in human diseases. Fanconi anemia-associated nuclease 1 (FAN1) participates in DNA repair. Thus cells with FAN1 mutation are more susceptible to DNA damage and arrested in late G2 phase.[12] Interestingly, patients carrying FAN1 mutation develop tubular atrophy and interstitial fibrosis.[12] In addition, G2/M arrested cells and DNA damage, assessed by the expression of γ-H2AX, correlate with fibrosis progression in kidney transplant recipients.[11]

Venkatachalam et al. suggest a variant of this pathogenic hypothesis on the mechanisms underlying maladaptive repair.[13] They propose that some tubular cells that dedifferentiate after ischemia/reperfusion-induced renal injury do not redifferentiate during recovery.[13] These abnormally undifferentiated epithelial cells may be present in any tubular segments, either in little cell clusters or as single, isolated cells.[13] Interestingly, this undifferentiated but growth-arrested epithelium is characterized by an intense signaling and produces and releases an array of fibrogenic mediators, despite being atrophic. This hypothesis is based on the observation that the signaling pathways required for dedifferentiation, migration, and proliferation are activated physiologically after AKI.[13] These cellular events are essential for regeneration and repair, but they should stop once tubules recover their structure and function. Thus the persistence of this activation signaling in undifferentiated cells is intrinsically pathologic and may explain the development of interstitial fibrosis featuring CKD.[13]

Recently, a particular attention has been dedicated to the tubular consequences of increased oxidative stress, featuring ischemia-reperfusion-induced AKI, and the subsequent mitochondrial changes. In normal conditions, mitochondria constantly undergo fission and fusion.[14] During cell injury, the dynamics are shifted toward the production of short mitochondria rods or spheres along with damage in the outer and inner membranes of the organelles, membrane leakage,

decreased function, and consequent cell death. Emerging evidence has suggested a pathogenic role of mitochondrial fragmentation in the maladaptive repair of AKI.[14,15] Funk and Schnellmann demonstrated that AKI is characterized by a persistent interference with mitochondrial homeostasis, resulting in a worsening in cellular respiration and a decline in intracellular ATP levels leading to tissue dysfunction and to the development of chronic damage.[16]

Endothelial Cells

Endothelial cells are considered one of the main targets in AKI, in particular after ischemia/reperfusion and sepsis-induced renal damage.[17] Studies in rats demonstrate that after ischemia/reperfusion injury, vascular functions are compromised for several days.[18] This period of time is characterized by an impaired autoregulation of blood flow, potentially caused by the inability of damaged/activated endothelial to synthesize nitric oxide.[19] Injured endothelial cells in peritubular capillaries may expose numerous surface proteins and receptors that promote the recruitment and adhesion of leukocytes and platelets, resulting in inflammation and further endothelial cell injury.[20,21] Also vascular permeability is altered, leading to interstitial edema, contributing to the reduction in microvascular perfusion. After apparent recovery of ischemia/reperfusion injury in the rat, there is a long-term reduction in peritubular capillary density, which precedes the development of visible fibrosis.[22,23] Renal hypoxia indeed is a key feature of chronic kidney damage and has been shown to contribute to its progression.[24]

A further interesting pathogenic process in the scenario of a maladaptive repair is represented by the endothelial-to-mesenchymal transition (EndMT), an extreme form of endothelial plasticity. EndMT first was described in embryonic heart development, but may appear also in the development of pathologic conditions, including cardiac fibrosis or the generation of carcinoma-associated fibroblasts.[25–27] In the last decade, several studies in animal models of CKD have suggested, for the first time, the contribution of EndMT in the progression of CKD-associated interstitial fibrosis.[28] In mice and swine models of ischemia/reperfusion injury, we, along with others, demonstrated that EndMT could be detected in the early phase of AKI.[29,30] Among the different factors involved in the progression of renal fibrosis, TGFβ is the most potent inducer of EndMT.[31] Echeverría et al. showed that endothelial cells exposed to LPS reproduce the phenotype of TGFβ-treated cells, highlighting the potential role of LPS to induce EndMT.[32] In line with this observation, we demonstrated the occurrence of EndMT in an animal model of sepsis-induced AKI.[33] In addition, our group reported that EndMT is an early event also in a swine model of ischemia/reperfusion-induced injury.[29] Indeed, after ischemia/reperfusion or LPS stimulation, endothelial cells lose their specific markers, CD31 and VE-cadherin, and acquire fibroblast markers, including α-SMA, vimentin, N-cadherin, and FSP-1. These transitioning cells detach from the intima of vascular wall and migrate into interstitial space differentiating in activated myofibroblasts and contributing to the deposition of extracellular matrix.[30]

Pericytes

Pericytes are contractile cells supporting the kidney microvasculature.[34] These cells of mesenchymal origin surround peritubular capillaries[34] and play a key role in maintaining the integrity of the microvasculature through an intimal connection with endothelial cells.[34] In response to AKI, pericytes may disengage from the vessel wall, promoting endothelial proliferation and migration.[35] This process, although primarily beneficial, may lead to an augmented capillary instability, ultimately causing microvasculature destruction and subsequent interstitial ischemia.[35,36] In addition, it has been proposed that pericytes may be directly involved in the development of interstitial fibrosis after AKI by migrating from their location and acquiring a myofibroblast phenotype.[6,37] Thus activated pericytes in AKI, breaking contact with endothelial cells and differentiating into myofibroblasts, may deliver a "double hit" of vascular instability and collagen deposition, both trademarks of progressive chronic renal damage.[35]

INNATE IMMUNE SYSTEM

Inflammation is a key feature of AKI and chronic progressive renal damage underlying CKD.[38] Thus it is conceivable that inflammation may represent the missing pathogenic link between these two conditions. The inflammatory response is characterized by the activation of the innate immune system, and in this scenario macrophages and the complement system play a pivotal role.

Macrophages

It is now clear that infiltrating monocyte-derived macrophages are essential in the process of tissue repair after injury.[38] Indeed, in the past few years, tissue macrophages have been recognized as orchestrators of repair in skin, muscle, gut, brain, and heart.[39] However, these cells can exhibit a significant flexibility in their phenotype and can acquire functional features that can induce tissue injury and fibrosis. These functional states are defined as M1 and M2 for proinflammatory and wound-healing phenotype, respectively.[39] In kidney diseases, macrophages have a bad reputation because they always have been considered as drivers of tissue injury through their proinflammatory and damaging abilities. Although this is certainly true, it seems that proinflammatory macrophages are *the exception that proves the rule.*[38] Indeed, the main function of the innate immune system is to clean up damaged tissues and promote repair without inducing further injury. Only severe tissue damage primes macrophages toward an injurious program.

Lee et al. demonstrated that ablation of macrophages at the onset of AKI is beneficial, significantly reducing kidney damage, whereas their ablation during the regenerative phase of AKI is harmful,[39] notably worsening tubular injury and promoting fibrosis. In addition, the infusion of macrophages with an M1 phenotype significantly exacerbated AKI, whereas the infusion of macrophages with an M2 phenotype did not.[39] Interestingly, when M1 macrophages are infused early after injury, they begin to express M2 markers with time, suggesting a phenotypic switch.[39] Thus, in the normal repair phase of AKI, macrophages may gain regenerative abilities. The identification of the mediators driving this phenotypic switch may be of great therapeutic relevance. In this perspective, several studies suggest the stimulation of macrophage Wnt signaling pathways in epithelium as a potential therapeutic option.[40] In fact, Lin et al. demonstrated that infiltrating macrophages in AKI produce and release Wnt7b, a Wnt ligand, that can induce tubular cells regeneration, promoting the recovery of renal

function and a reduction in interstitial fibrosis.[40] Although the activation of Wnt/β-catenin appears to be renoprotective in AKI, the same molecules have been reported to drive CKD onset and progression.[41] Transitory activation of Wnt/β-catenin induces tubular cells regeneration and prevents the development of interstitial fibroblasts, whereas prolonged activation of the same signaling machinery may prime a maladaptive repair.[41] This hypothesis is supported by the observation that sustained activation of Wnt/β-catenin, through overexpression of Wnt1, accelerates the transition from AKI to CKD, whereas inhibition of Wnt/β-catenin avoids AKI to CKD progression.[41]

Recently, Lech et al. demonstrated that tumor necrosis factor-alpha (TNF-α) may play a significant role in this context using mice lacking IL-1 receptor–associated kinase M (IRAK-M).[42] IRAK-M is an inactive kinase that antagonizes proinflammatory signaling in macrophages. Interestingly, the lack of IRAK-M does not influence the extent of renal injury, but although wild type mice present a normal reparative response, IRAK-M knockout mice produce an increased amount of TNF-α and show a significant increase in renal fibrosis and tubular atrophy after injury.[42] The infusion of the TNF-α inhibitor etanercept significantly reduces tubular atrophy and interstitial fibrosis.[42]

Complement

The complement system plays a major role in the defense against pathogens as well as in the clearance of apoptotic and necrotic cells.[43] This enzymatic cascade can be activated through three different pathways: the classical, the lectin, and the alternative pathways.[43] The classic pathway is primed by the binding of C1q to immunoglobulin, acute phase proteins, apoptotic bodies, or necrotic debris. The lectin pathway is activated by the interaction between Mannan-binding lectin (MBL) and carbohydrate ligands usually present on the bacterial wall or IgA. The alternative pathway is constantly active at low level through the nonregulated hydrolysis of C3. A growing body of evidence suggests a pivotal role of complement activation in the pathogenesis of ischemia/reperfusion-induced renal injury.[43,44] Mice deficient in C3 and Factor B, the two main factors of the alternative pathway, are protected from AKI.[45–47] Our group reported the deposition of C1q, MBL, and C4d within peritubular capillaries in a swine model of ischemia/reperfusion injury and in graft biopsies of patients with delayed graft function, a form of posttransplant AKI depending on ischemia/reperfusion.[48] Interestingly, in addition to its well-defined role in the pathogenesis of acute renal injury, complement may play a primary role also in the repair process and, in particular, in the maladaptive repair leading to CKD. In our experimental model of ischemia/reperfusion-induced AKI, the inhibition of the classical and lectin pathways by the infusion of C1 inhibitor significantly reduced the development of interstitial fibrosis and the appearance of interstitial myofibroblast.[29] In vitro both anaphylotoxins, C3a and C5a, can induce EndMT, leading to a profibrotic phenotype of endothelial cells.[29]

ADAPTIVE IMMUNE SYSTEM

It is now well established that adaptive immune response plays a key role in the pathogenesis of AKI. Different immune cells, including dendritic cells, NK T cells, and T and B lymphocytes have been shown to be directly or indirectly involved.[49–51] Conventional $CD4^+$ lymphocytes are well established to participate in early injury, whereas several observations suggest that $CD4^+CD25^+FoxP3^+$ regulatory T cells may be protective.[49–51] In addition to B and T lymphocytes' role in the pathogenesis of acute tubulointerstitial injury, there is now an increasing body of evidence suggesting a role for these immune cells in the regulation of the balance between adaptive and maladaptive repair. Jang et al. demonstrated that adoptive transfer of B cells into mature B cells–deficient mice, at the time of ischemia/reperfusion, reduced tubular proliferation and increased tubular atrophy, suggesting that B cells themselves may inhibit an appropriate tissue repair.[52] On the other hand, Burne-Taney et al. reported that lymphocytes of animals previously exposed to severe ischemia/reperfusion injury can induce albuminuria after transfer to naive recipients, indicating that immunologic memory may contribute to the development of proteinuria after AKI, and this proteinuria may have secondary effects to facilitate the onset of CKD.[53]

Senescence: the Bridge Between AKI and CKD

Aging is an important risk factor for the development of AKI as well as for the progression of the acute damage into a chronic progressive injury.[54] There is an increasing body of evidence suggesting that AKI-induced cellular and tissue senescence may represent a key pathogenic step in maladaptive repair linking AKI with CKD.[54] Indeed, the tubular cell phenotype and the characteristic inflammatory response featuring the maladaptive repair clearly suggest the acceleration of a physiologic senescence process.[54] Thus accelerated cell and tissue senescence as the main pathogenic mechanism of maladaptive repair may represent a unifying hypothesis. The mechanisms of cellular and tissue aging are still poorly understood, although the role of different signaling proteins recently has been suggested in this scenario.

Activation of prosenescent signaling molecules including p21 and p16*INK4a*, with the subsequent cell cycle arrest, caused by the inhibition of Rb phosphorylation, characterizes cellular senescence.[55] Notch1 recently has been proposed as a key effector in the pathogenesis of cellular senescence leading to p21 and p16INK4a expression in several cell lines.[56,57] The Notch signaling pathway, consisting of several receptors, Notch1 through 4, and several ligands,[58] modulates adult tissue homeostasis and regeneration.[59] Because receptors and ligands are transmembrane proteins, a direct cell-cell contact is necessary to activate downstream signaling events.[59] Upon activation, Notch receptors undergo proteolysis with the subsequent release of the intracellular domain of the receptor.[60] The Notch intracellular domain (NICD) translocates to the nucleus and induces the transcription of several target genes.[60] In embryonic kidney, Notch regulates nephron segmentation, although in the adult kidney, Notch activation is significantly limited.[61] Kobayashi et al. reported that Notch signaling is reactivated in a model of ischemia/reperfusion-induced AKI.[62] They speculated that Notch may influence tubular epithelial cellular proliferation. This hypothesis is supported by the observation that in a similar model of AKI infusion a Notch ligand enhances renal repair.[63] Although Notch seems pivotal in tubular regeneration, there is also evidence that in a chronic setting its activation can induce interstitial fibrosis.[64] Indeed, conditional NICD expression can worsen tubular cell injury and promote interstitial fibrosis, along with the expression of cellular senescence markers, p21 and p16*INK4a*, whereas pharmacologic inhibition of Notch significantly reduces

tubulointerstitial injury and antagonizes AKI-induced prosenescent pathway activation.[58]

α-Klotho (Klotho) is a well-known anti-aging factor.[65] Klotho knockout mice present a premature aging syndrome characterized by accelerated thymic involution, skin atrophy, sarcopenia, vascular calcification, osteopenia, cognition impairment, and motor neuron degeneration, among others. On the other hand, mice overexpressing Klotho present a significant increase in their life span.[65] Klotho may be expressed as a transmembrane protein consisting of an N-terminal domain, an extracellular domain with two internal repeats (KL1 and KL2), a single transmembrane domain, and a short intracellular domain.[66] Klotho expressed on the cell membrane of tubular cells acts as a coreceptor for fibroblast growth factor 23 (FGF23), a bone-derived peptide that induces phosphaturia through the inhibition of phosphate reabsorption and inhibits vitamin D hydroxylation in the kidney.[67] On the other hand, Klotho may be expressed as a soluble protein secreted into blood and urine. Soluble Klotho has been shown to modulate the activity of ion channels and growth factor receptors, most likely through its sialidase activity.[68] All major tubular segments along the nephron express membrane-bound Klotho,[69] and the kidney plays a central role in the maintenance of a soluble Klotho homeostasis contributing to its release and clearance.[70]

Animal models clearly showed a downregulation of Klotho expression in AKI from a variety of causes including ischemia/reperfusion, ureteric obstruction, lipopolysaccharide infusion, hypovolemia, and nephrotoxins.[71,72] Murine model of bilateral ischemia/reperfusion-induced injury showed a remarkable reduction in the Klotho mRNA and protein expression.[73] In addition, our group observed a significant reduction of renal Klotho expression in patients with delayed graft function, a form of ischemia/reperfusion-induced AKI. Several proinflammatory mediators including TNF-α and interferon-gamma can reduce Klotho in vitro and in vivo, suggesting a role for the inflammatory response featuring AKI in the reduction of Klotho expression.[74,75] We recently suggested that the downregulation of Klotho may be linked to the activation of the complement cascade.[76] Indeed C5a significantly reduce Klotho expression in cultured tubular epithelial cells. In addition, complement cascade inhibition by C1 inhibitor infusion in a swine model of ischemia/reperfusion-induced AKI can preserve tubular Klotho expression.[76] The reduced expression of this antiaging molecule may have a pathogenic relevance in the repair process. Indeed ischemia/reperfusion cause a more severe renal injury in heterozygous Klotho haplo-insufficient compared with wild-type animal, whereas transgenic mice overexpressing Klotho appear to be protected. In addition, the administration of Klotho either directly or through the infection with an adenovirus harboring the mouse Klotho cDNA stimulates renal repair from AKI.[73] In consideration of its antiaging effect, it is conceivable that the beneficial effects of Klotho in the healing phase of AKI may be due to its ability to slow the accelerated senescence featuring maladaptive repair in this setting.[77]

Maladaptive Repair as a Therapeutic Target

All the information gathered in the last decade on the molecular and cellular pathways leading from AKI to CKD, although still largely incomplete, may have a significant clinical relevance, potentially suggesting new therapeutic targets to prevent the maladaptive repair featuring a relevant percentage of patients with AKI. Interestingly, most of the pathways described are targets of existing drugs already used for other indications, and this detail may facilitate interventional studies. Indeed, biologic agents that interfere with the complement cascade, including eculizumab, an anti-C5 monoclonal antibody, and the recombinant C1 inhibitor, are already registered for other pathologic conditions. There are ongoing clinical trials to prove their efficacy in the prevention of delayed graft function, a posttransplant form of AKI.[78–80] However, as in other key processes, the pathways regulating normal and maladaptive renal repair demonstrate a high rate of redundancy. Thus hitting a single target may not give the expected results, and we should consider a multidrug approach.

Key Points

1. Maladaptive repair represents the link between acute kidney injury (AKI) and chronic kidney disease.
2. Resident cells, in particular endothelial cells, are key players in this scenario.
3. Both arms of the immune system, innate and adaptive, interact with resident cells to modulate their activation.
4. AKI-induced cellular and tissue senescence represents the final and common pathogenic step in maladaptive repair.

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A complete reference list can be found online at ExpertConsult.com.

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