Abstract

Septic acute kidney injury (AKI) is the most common form of AKI seen in critically ill patients in developed countries. Its pathogenesis has been traditionally attributed to ischemia secondary to decreased cardiac output and hypotension, which trigger sustained renal vasoconstriction and in turn exacerbate and sustain the ischemia. This paradigm is supported by the fact that many patients who develop AKI do so in the setting of hemodynamic instability and also by evidence that renal blood flow is decreased and renal vascular resistance increased when they are measured in patients with AKI. However, recent evidence shows that renal blood flow may vary from increased in some animal models to normal in some patients and to decreased in other patients. Furthermore, the induction of prolonged severe subtotal ischemia by acute occlusion of the renal artery does not seem to trigger subsequent renal vasoconstriction and, finally, experimental studies suggest that immune-mediated injury may be a more likely cause of tubular cell dysfunction than ischemia. These lines of evidence suggest that the pathogenesis of AKI is complex, does not simply involve ischemia, and may differ according to the etiological trigger.
from major surgery to drug toxicity. As such, AKI seems to represent the final common pathway of different types of injury suggesting that the mechanisms involved are essentially the same in almost all cases: decreased renal blood flow, associated renal vasoconstriction in response to decreased perfusion, tubular cell hypoxia, bioenergetic failure and cell death (acute tubular necrosis (ATN)). Septic AKI, however, seems unlikely to follow this pattern because, under most circumstances, glomerular filtration rate (GFR) decreases rapidly, despite increased cardiac output and an adequate mean blood pressure, which is supported by vasopressor drugs. These observations have led to recent challenges to the concept that ischemic tubular ischemia is responsible for septic AKI. In this article, we will review recent evidence that challenges the traditional view of septic AKI as a condition mostly, if not wholly, secondary to ischemia.

The Ischemia Paradigm

The main cause of septic AKI has been assumed to be ATN caused by hemodynamic instability and subsequent renal vascular vasoconstriction and kidney ischemia [1]. Renal vascular vasoconstriction triggered by hemodynamic instability then exacerbates and sustains renal ischemia and eventually causes established ATN. In response to this pathophysiologic paradigm, over the last 40 years or more, all efforts to prevent AKI or reverse it have been based on a logically related therapeutic paradigm. This therapeutic paradigm states that preservation of ‘adequate’ perfusion or rapid correction of hypoperfusion by fluid resuscitation and/or vasoactive drugs, and/or the correction of renal vasoconstriction by the administration of renal vasodilators like low-dose dopamine, is the only logical approach to the prevention and treatment of AKI. Unfortunately, all of these strategies, although widely applied, have so far failed to achieve any demonstrable success. This failure may, of course, reflect problems other than an incorrect understanding of the pathogenesis of AKI. For example, failure may have been due to the use of drugs that are not sufficiently effective, the application of interventions too late in the course of AKI to be able to reverse those biological processes responsible for cell injury, the insufficient correction of hypoperfusion, or any combination of the above factors. Another possible explanation for our therapeutic failures is that the renal ischemia paradigm is only partly correct and can only explain a small part of what might be happening during AKI, especially in sepsis. When considering a challenge to a 40- to 50-year-old paradigm, one needs to consider the available evidence as objectively as possible. One initial step in direction of challenging the ischemia-AKI paradigm might come from a discussion of what evidence exists that ATN is the histopathological substrate of septic AKI.
Histopathological Changes in Septic AKI

Light Microscopy in Humans

Recently, Langenberg et al. [2] performed a systematic review of the histopathology of septic AKI. They found that only 22% of all 184 patients with AKI, whose histopathology had been reported in the literature, actually showed signs of ATN on biopsy or post-mortem. There are two additional detailed human studies (beyond those considered by Langenberg) which seem to have examined the histopathology of septic AKI. First, Brun and Munck [3] examined histopathological changes of 33 patients with acute renal failure (ARF) following shock including sepsis by biopsy or necropsy material. ATN findings (predominantly in distal tubules) were observed in only 5 of the 33 patients. So the authors concluded the most striking feature of AKI was that moderate structural changes contrasted sharply with complete functional breakdown. Second, Diaz de Leon et al. [4] reported that ATN findings were observed in only 20 of 40 patients with septic AKI. Unfortunately, the details of histopathology were not described in this article. Even if these articles are added to Langenberg’s systematic review, only 25% of all patients with septic AKI reported in the literature show ATN findings.

More recently, Lerolle et al. [5] reported the histopathological findings in 19 patients who died from severe sepsis shock in the intensive care unit. They found that all patients had changes consistent with ATN. They also found that apoptosis was a common finding with approximately 3% of cells examined showing evidence of apoptotic changes. There is no information on the presence of apoptosis in normal human kidneys. Even when these highly biased cases (the most severe end of the spectrum with sustained septic shock leading to death as well as AKI) are included in our analysis, only a minority of patients with septic AKI appear to have ATN as the histological substrate for their functional loss. If the paradigm is that ischemia leads to ATN, which then leads to AKI, then this paradigm is clearly not adequately supported, because about 70% of patients with sepsis as AKI appear not to have ATN. Thus, in the severest cases, this paradigm may have some credence, but in most patients with septic AKI, it does not.

In animal experiments, which model sepsis to study its renal effects, the findings are similar. Langenberg et al. [2] also performed a systematic review of animal model of septic AKI using all articles that make specific mention of histopathology. The authors reported that only 23% in all studies showed evidence of ATN, a finding which is remarkably consistent with the human data.

Thus in most cases of human and experimental septic AKI there appears to be a degree of dissociation between the marked loss of GFR and urine output, which often decreases to almost zero, and the histopathologic changes which are surprisingly limited in most patients. This dissociation remains unexplained. Equally important, the major functional event of septic AKI (loss of GFR) would
logically lead to a focus on the glomerulus as a potential site of dysfunction and yet essentially all literature has focused on the tubules instead. Despite these concerns, it is possible that injury occurs to both tubules and glomeruli and we simply cannot see it with light microscopy. Electron microscopy might shed some light on this subject.

**Electron Microscopy**

There are no reports or case series of patients with septic AKI to describe the electron microscopy findings of this condition. Thus, there is no knowledge of whether tubular injury occurs at a level that is potentially clinically significant but not detectable with light microscopy. However, experimentally, cytokines (TNF-\(\alpha\), IL-1\(\alpha\), IFN-\(\gamma\)) can induce shedding of viable, apoptotic and necrotic proximal tubule epithelial cells. This process appears dependent on nitric oxide and not on ischemia. Cytokine administration can also change the morphologic features of proximal tubule epithelial cells. Finally, plasma of burns septic AKI patients can induce alteration in the distribution of cytoskeleton actin fibers in tubular cells [6]. These observations are important because they suggest that tubular injury in septic or inflammatory states may be mediated by immunological injury, not ischemia. Knowledge that this can happen experimentally, and our inability to exclude immunological injury in humans, raise the issue of whether septic AKI is a form of organ injury triggered by the innate immune response to infection. Moreover, given this experimental evidence that cytokines can induce tubular cell injury, given our knowledge that sepsis is characterized by a cytokine storm, given that there is no reliable evidence of ischemic ATN in the majority of animal experiments of sepsis or in humans with sepsis, given that most septic patients have a hyperdynamic circulation with a high cardiac output, and given that recent animal models of such hyperdynamic sepsis show increased rather than decreased renal blood flow, why do we think that septic AKI is due to ischemia instead of immune injury? How can we exclude immune-mediated injury as a cause of septic AKI? One related consideration is that, under many circumstances, immune-mediated cell injury takes the form of apoptosis. This invites interest in considering what role apoptosis may play in the development of septic AKI.

**Apoptosis of Kidney Tubules in Septic AKI**

In one report of early autopsy findings organs were examined for apoptosis in 20 septic patients [7]. In this study, despite the high prevalence of clinical renal dysfunction (65%) in patients with sepsis, only 1 septic patient had evidence of kidney necrosis. No renal tubular apoptosis was seen in any septic patient by light microscopy. The authors relied heavily on use of fluorescent terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) labeling. TUNEL does not always label apoptotic cells and there is often staining of nuclei that show no
morphological evidence of apoptosis [8] and thus, it remains unclear to what extent apoptosis may or may not play a role in septic AKI in humans.

The authors of the above study once again described that renal histology did not reflect the severity of renal injury indicated by the decrease in kidney function. The TUNEL method could not discriminate between septic patients and control patients. Importantly, it was shown that with the TUNEL method, a delay in tissue fixation caused a marked increase in the number of apoptotic-positive cells at 3 and 6 h compared with immediate fixation in almost all organs. More recently, another case series examined the post-mortem findings in a cohort of patients who died of septic shock [5]. In this study the major finding was that of ATN in all cases. However, apoptosis was also seen as prominent with 3% of all cells showing evidence of apoptotic injury. The cause of such injury remains unclear and it is also uncertain whether such degree of apoptosis has pathogenetic significance or simply represents an epiphenomenon. Similarly, although it is known that plasma from the septic burns patients with septic AKI induced a pro-apoptotic effect in tubular cells in vitro [6], the clinical significance of apoptosis as a contributor to loss of GFR in AKI remains unknown.

In a model of sepsis, Messaris et al. [9] followed the time course of apoptosis using cecal ligation and puncture (CLP) in rats. The time distribution of all types of cell death was increased significantly 6 h after the induction of sepsis, and declined subsequently. The cells initiating apoptosis were significantly more common at 6 than at 48 h post-CLP. On the other hand, other studies report no apoptotic cells detected in the kidney after CL. Hotchkiss et al. [7] also examined whether apoptosis occurs systemically in lymphoid and parenchymal cells in CLP of mice. They found that the extent of apoptosis was less in kidney compared with other organs such as lymph nodes and spleen. In sepsis models using lipopolysaccharide (LPS) injection, the time course of apoptosis peaks at 8–12 h and is still apparent after 48 h. In some studies, the amount of apoptosis in individual animals correlated with the extent of renal functional impairment, suggesting that apoptosis may be intimately involved in LPS-induced ARF or be a good marker of other mechanisms responsible for loss of GFR. On the other hand, several other studies reported no apoptotic cell 16 h after LPS injection.

Caspases are essential proteases for initiation and execution of apoptosis and for the processing and maturation of the inflammatory cytokines IL-1β and IL-18, and plasma of burns patients with septic AKI increases the activity of caspases-3, -8 and -9 in tubular cells [6]. Broad-spectrum caspase inhibitors such as Z-VAD-FMK can prevent lymphocyte apoptosis in sepsis, and in turn, improve septic animal survival by 40–45%. However, at high doses, caspase inhibitors exacerbated TNF toxicity by enhancing oxidative stress and mitochondrial damage.

It must be noted that in the normal mature adult, there is a balance between normal levels of mitosis and apoptosis, so that individual organs remain the same size. In the conditions like sepsis, however, toxic cytokines may tip the balance
towards more apoptosis than mitosis. Even when the number of apoptotic cells detected in any experiment is not great, this needs to be seen in the light of the rapidity of the apoptotic process, where cells may die, be phagocytosed and the cellular content broken down via lysosomal enzymes and recycled in just a few hours. Even if accumulating apoptotic cell death resulted in cellular loss of up to 50%, the percentage of dying cells identifiable at a single time point could easily be less than a few percent of the entire tissue mass, especially when this atrophic process occurs over a span of weeks [10]. Thus, finding that only a small percentage of tubular cells are apoptotic at the time of sampling cannot exclude a very significant destructive process. In our experimental animals with sepsis induced by *Escherichia coli* infusion, apoptosis is relatively common (fig. 1, 2).

Further evidence against major histopathological changes in sepsis is the ability of the kidney to recover function rapidly after experimental sepsis [11]. This suggests that there is not any major damage to the glomeruli or tubules due to cell death from either necrosis or apoptosis in sepsis.

### Role of Toll-Like Receptors

Innate immunity is first line of host defenses for the pathogens. It recognizes pathogens via Toll-like receptors (TLRs), which detect specific molecular

**Fig. 1.** Detection of apoptosis by TUNEL technique in kidney from septic sheep following infusion of live *E. coli*. The dark staining nuclei indicate the presence of apoptosis (as shown by arrow).
patterns of pathogens from Gram-negative to Gram-positive organisms to fungi, viruses or parasites. TLRs are thought the main initial modulators of the inflammatory cascade associated with a pathogen attack [12]. So far, at least 11 members of the TLR family have been found in mammals, and TLR2, 4 and 9 appeared particularly important in septic AKI. For example, TLRs 1–10 have all been detected in human kidney cells by several methods including polymerase chain reaction, in situ hybridization, and immunohistochemistry [12]. TLR4 mutations and polymorphisms are reported in human kidney cells.

Using TLR knockout mice, one can understand the possible role of TLRs in the pathophysiology of septic AKI. The C3H/HeJ strain of mice that lack the function of TLR4 are resistant to LPS-induced septic AKI and mortality [13]. These experimental findings are crucial because they further suggest a powerful role by the immune system in the pathogenesis of septic AKI. If this is the case in man, the ischemia-AKI paradigm is seriously challenged.

In a CLP model, monoclonal antibody for TLR4 and myeloid differentiation protein-2 complex improve survival [14]. Myeloid differentiation factor 88 is a main messenger molecule for TLRs, acting as a link between the receptors and downstream kinases such as NF-κB and TNF. Myeloid differentiation factor 88 knockout mice do not develop septic AKI or show the histopathological changes of AKI after CLP. Yasuda et al. [15] recently reported the protective

---

Fig. 2. Detection of apoptosis by TUNEL technique in kidney from septic sheep following infusion of live E. coli during the recovery phase. Medullary apoptosis is indicated by the dark staining cells (as shown by arrow).
effect of chloroquine, an inhibitor of TLR9, in preventing septic AKI in a CPL model.

After recognition of pathogen by TLRs, pro-inflammatory cytokines (TNF, IL-6, IL-8) are released into the circulation within the first several hours. First, TNF-α and IL-1β are released and, later, IL-6, IL-10 and nitric oxide. Chawal et al. [16] reported that an increased IL-6 level is a significant risk factor for septic AKI, further supporting the notion that inflammation is a significant component of septic AKI. Elevated soluble TNF receptor level is an independent predictor of mortality among patients developing septic ARF [17].

TNF-α directly injures kidney tubules, independent of inducible nitric oxide synthase, hypotension, apoptosis, and morphologic alterations. High-dose TNF-α causes renal tubular necrosis. Low or moderate doses of TNF cause glomerular inflammation, but no histological change in tubules.

TNF receptors (TNFR) are needed to mediate the injurious effects of TNF on kidney cells. TNFR1+/+ kidneys transplanted into TNFR1–/– mice develop severe ARF after LPS injection, but TNFR1–/– kidneys transplanted into TNFR1+/+ mice do not. Therefore, TNF is a key mediator of LPS-induced ARF, acting through its receptor TNFRI in the kidney.

Mitochondrial Dysfunction and ATP Depletion

ATP depletion can cause either necrosis or apoptosis in mouse proximal tubular cells in vitro. A legitimate question, if ischemia is the cause of septic tubular injury, is to ask whether mitochondrial function is lost in septic AKI, especially because the determination on whether cells die by either necrosis or apoptosis depends on the depletion of ATP. However, continuous infusion of LPS (0.4 μg/kg/h) did not change renal blood flow, renal mitochondrial respiration, and renal lactate/pyruvate ratio [18].

Magnetic resonance imaging (MRI) studies by May et al. [19] demonstrated no change in total ATP or β-ATP/total ATP ratio in the kidney during hyperdynamic sepsis despite profound hypotension and anuria. Furthermore, Dear et al. [20] performed MRI with gadolinium-based G4 dendrimer intravenous contrast in CLP mice. 24 h post-CLP, aged mice had a distinct pattern of renal injury that was different from renal injury induced by either ischemia reperfusion or pre-renal azotemia. Moreover, MRI detected renal dysfunction 6 h post-CLP, a time when serum creatinine was still normal.

Conclusions

The ischemia-ATN paradigm is flawed as an explanation of tubular injury in septic AKI. ATN is not the most common histopathological finding in septic
AKI and only occurs in a third of cases. Apoptosis may be a more important process than previously appreciated. A large body of experimental data supports the notion that the innate immune system is deeply involved in the pathogenesis of septic AKI in a way that is independent of decreased renal perfusion. Furthermore, magnetic resonance spectroscopy shows preserved levels of ATP in severe septic shock and a pattern of injury which is unique in severe sepsis. Our understanding of the pathogenesis of tubular injury in septic AKI is limited. Until it is sufficiently increased, therapeutic strategies will continue to fail.

References


