The pathogenesis of septic acute renal failure

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Purpose of review

Acute renal failure is a serious condition that affects as many as 20% of ICU patients. The most common causes of acute renal failure in the ICU patient are severe sepsis and septic shock. The mortality of acute renal failure in septic critically ill patients remains high despite our increasing ability to support vital organs. This is partly the result of our poor understanding of the pathogenesis of sepsis-induced renal dysfunction. Accordingly, a review of our current understanding of the pathogenesis of septic acute renal failure is timely and relevant.

Recent findings

Throughout the past half century, acute renal failure of acute illness has essentially been considered a hemodynamic disease caused by kidney ischemia, a view derived by findings in animal models. Unfortunately most such models are greatly deficient in that they do not reproduce the high cardiac output, low systemic vascular resistance state typically seen during human sepsis. Furthermore, most models inducing so-called acute tubular necrosis are based on ischemia–reperfusion (renal artery clamping), an event with little relevance to human sepsis. Recent research highlights a new possible and emerging concept for the pathogenesis of septic acute renal failure: acute apoptosis. This concepts fits well with the typical paucity of histologic changes seen in so-called acute tubular necrosis and with growing evidence of a role for apoptosis in organ injury during sepsis and inflammation in general. Furthermore, the authors present evidence that some potential treatments recently shown to affect the mortality of critically ill patients, (activated protein C, intensive insulin treatment, and low-volume mechanical ventilation) might have antiapoptotic activity.

Summary

This review suggests that, on the evidence available, septic acute renal failure is more likely to be an immune or toxic state rather than simply a hemodynamic condition. The authors speculate that future insights into its pathogenesis might lead to a paradigm shift away from the concept of acute tubular necrosis, which has never been convincingly shown in sepsis, to that of acute tubular apoptosis.

Keywords

acute renal failure, sepsis, acute apoptosis, activated protein C

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Abbreviations

APC	activated protein C
ARF	acute renal failure
LPS	lipopolysaccharide
TNF	tumor necrosis factor

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Introduction

Acute renal failure (ARF) affects 5 to 7% of all hospitalized patients [1–3]. Sepsis and septic shock remain the most important cause of ARF in critically ill patients, and account for more than 50% of cases of ARF in the ICU [4–6]. Despite our increasing ability to support vital organs and resuscitate patients, the incidence and mortality of septic ARF remain high [5,6]. A possible explanation of why, despite treatment, ARF is so common in severe sepsis and septic shock and why mortality has remained high might relate to our minimal understanding of septic ARF and its pathogenesis. It is therefore very important for critical care physicians to have an appreciation of what is known and not known about this condition to implement rational therapies. In this article we review current knowledge in the field and highlight new developments in our understanding of septic ARF.

Pathogenesis

Our understanding of the pathogenesis of human ARF in general is markedly affected by the lack of histopathologic information of what happens to the human kidney as glomerular filtration rate decreases and oliguria develops in a variety of clinical settings. This lack of information stems from the risks associated with renal biopsy (especially repeated renal biopsy), which make it ethically unjustifiable to obtain tissue from patients who do not have suspected parenchymal disorders and whose ARF is considered secondary to so-called "prerenal" factors. In the absence of such information, we rely on indirect assessments of what might be happening to the kidney. Such assessments are based on blood tests and urine tests, and they force us to "guess" what might be happening to kidney cells by using "smoke screens and mirrors." It is not surprising, therefore, that our under-

standing of septic human ARF has advanced very little during the last 50 years. To overcome such limitations, animal models of ARF have been developed that enable more sophisticated and invasive measurements to be made. Unfortunately, as recently highlighted [7•], these animal models have been mostly based on ischemia–reperfusion injury or drug-induced injury. Neither model is relevant to septic ARF, and information obtained from such models may be misleading when applied by clinicians to interpret what might be happening to a septic patient who is developing ARF in the ICU.

The primacy of renal blood flow

A major paradigm that has been derived from observations in animals and humans with hypodynamic shock (hemorrhagic, cardiogenic, or even septic) is that ARF is the result of renal ischemia. This construct implies that restoration of adequate renal blood flow should therefore be the primary means of renal protection in critically ill patients. Whether, in the presence of a normal or increased cardiac output, renal blood flow actually decreases significantly or remains stable or even increases remains controversial.

In several experimental studies of septic ARF, global renal blood flow declines after induction of sepsis or endotoxemia [8,9]. This may result not only in a reduction in glomerular filtration but also, if hypoperfusion is severe and prolonged, in metabolic deterioration and diminished contents of high-energy phosphates, possibly causing cells death, acute tubular necrosis, and ARF.

On the other hand, other studies show that the renal circulation participates in the systemic vasodilatation seen during severe sepsis/septic shock, so that renal blood flow and glomerular filtration do not diminish, and the development of septic ARF occurs not in the setting of renal hypoperfusion but in the setting of adequate and even increased renal perfusion. Ravikant and Lucas [10], for example, studied a porcine model of sepsis and showed that during hyperdynamic sepsis there was an increase in global renal blood flow and an increase in medullary blood flow. Brenner *et al.* [11] developed and studied a percutaneously placed thermodilution renal blood flow catheter in eight critically ill patients. They demonstrated that sepsis-induced acute renal dysfunction occurred despite normal values of total renal blood flow. Furthermore, during human sepsis, patients typically show a hyperdynamic state. Observations in hyperdynamic models of sepsis may, therefore, be much more relevant to human septic shock. Indeed, the reason why the results of experimental studies are so different in terms of renal perfusion may be entirely related to the animal models (including animal type and type of insult), different methods of measurement, the time and frequency of measurements and, more important, the state of the systemic circulation (hypodynamic or hyperdynamic state). In fact, the consistent observation is that once a hyperdynamic state exists, global renal hypoperfusion/ischemia is *not* the norm.

A comprehensive review of electronic reference libraries, using sepsis and acute renal failure as the key words and limited to animal models, found 39 original articles. Of these, only 10 reported the cardiac output and only five were conducted in the setting of increased cardiac output. Furthermore, only six measured systemic vascular resistance and only three showed a decrease in systemic vascular resistance (the typical hemodynamic state of sepsis in the ICU). Because the more reliable hemodynamic measurements similar to those obtained in humans come from large-animal experiments, it is worth focusing on such studies. There are only two such studies, which measured and reported an increased cardiac output. They show no evidence of renal hypoperfusion. All these observations demonstrate that there is a significant lack of relevant animal models that simulate the hemodynamic state of septic patients and would thus be relevant in helping us advance our understanding of ARF. To put it bluntly, we simply do not know what happens to renal blood flow in hyperdynamic human sepsis.

In conclusion, renal hypoperfusion might be important in hypodynamic states but is unlikely to play a key role in the development of ARF during hyperdynamic sepsis (the state seen in the vast majority of critically ill, septic patients with severe ARF).

Intrarenal hemodynamic change

It is possible that, even though there is preserved or increased global renal blood flow in hyperdynamic sepsis, internal redistribution of blood flow favoring the cortex may occur [12]. Unfortunately, no studies have looked at medullary and cortical blood flow in hyperdynamic sepsis with technology that allows continued measurement over time. A recent investigation by our group used laser Doppler flowmetry to monitor medullary and cortical flow continuously in hyperdynamic septic sheep [13••]. We found that both flows remain unchanged and that the administration of vasopressor therapy (norepinephrine) induced a significant increase in such flows. These observations challenge the view that the medulla is ischemic during hyperdynamic sepsis but simultaneously highlight that hemodynamic factors are indeed at work, which can be modified by interventions that affect systemic blood pressure and cardiac output. Thus, intrarenal hemodynamic events do occur that might affect function. However, their favorable modification by vasoconstrictor therapy challenges the widely held view of what is optimal renal resuscitation in sepsis. Furthermore, even though hemodynamic changes might be important, they are likely to represent only some of the mechanisms responsible for loss of function. Using the

same model, the activation of apoptotic mechanisms can also be shown (unpublished data).

Nonhemodynamic injury

From the previous discussion we know that neither global renal hemodynamic changes nor intrarenal hemodynamic changes can be consistently shown to be the sole contributor to sepsis-induced ARF. There must, therefore, be other mechanisms at work that are not hemodynamic in nature. These factors that contribute to ARF in sepsis might be immunologic or toxic in nature.

Sepsis is characterized by the release of a vast array of inflammatory cytokines, arachidonate metabolites, vasoactive substances, thrombogenic agents, and other biologically active mediators. A large body of experimental data suggests that these various mediators and neuroendocrine mechanisms might be involved in the pathogenesis of organ dysfunction in sepsis [14•].

Tumor necrosis factor and acute renal failure

Tumor necrosis factor (TNF) has been demonstrated to play a major role in the pathogenesis of Gram-negative septic shock, mediating a broad spectrum of host responses to endotoxemia. In the kidney, endotoxin stimulates release of TNF from glomerular mesangial cells [15]. More recently the direct toxic role of TNF to the kidney has been become clear. Knotek *et al.* [16•], using TNFsRp55-based neutralization of TNF, achieved protection against lipopolysaccharide (LPS)-induced renal failure in wild-type mice. With pretreatment using TNFsRp55, glomerular filtration rate decreased by only 30%, compared with a 75% decrease without TNF neutralization, suggesting that TNF plays an important role in septic ARF. Furthermore, van Lanschot *et al.* [17] infused TNF in sublethal doses in dogs. TNF induced an increase in water and sodium excretion, an effect that could be prevented by prior cyclooxygenase inhibition, suggesting that vasodilatory prostaglandins mediated some of the renal response to sublethal TNF in this model. Cunningham *et al.* [18••] used *Escherichia coli* LPS as an intraperitoneal injection to establish a mice model of sepsis, and showed that LPS-induced ARF can be attributed to TNF acting directly on its receptor, TNFR1, in the kidney. Mice deficient in TNF receptor were resistant to LPS-induced renal failure, had less tubular apoptosis, and had fewer infiltrating neutrophils. Although TNF receptor-positive kidneys transplanted in TNF receptor-negative mice developed LPS-induced renal failure, TNF receptor-negative kidney implanted in TNF-positive mice did not. TNF thus seems to be an important direct mediator of endotoxin's effects during sepsis-induced ARF. These observations suggest that toxic/immunologic mechanisms are important in mediating renal injury during sepsis and that hemodynamic factors do not operate in isolation and may not even be of major importance.

A new concept of acute renal failure in sepsis: apoptosis

Apoptosis is a form of cell death that is mediated by a genetically determined biochemical pathway and is characterized morphologically by cell shrinkage, plasma membrane blebbing, chromatin condensation, and nuclear fragmentation [19,20,21•,22]. Cells can die by one of two pathways: necrosis or apoptosis. Necrosis results from severe ATP depletion and leads to rapid, uncoordinated collapse of cellular homeostasis. Apoptosis is an energy-requiring and genetically directed process. Morphologic differences between necrosis and apoptosis are summarized in Table 1.

There is now strong evidence to show that human renal tubular cells die by apoptosis as well as necrosis in experimental models of acute ischemic and toxic renal injury [20,21•,22]. Endothelial cells can undergo apoptosis in response to a variety of stimuli. The potential causes of apoptosis are as follows:

- Growth factor deficiency
  - Nerve growth factor
  - Epidermal growth factor
  - Insulinlike growth factor-1
- Loss of cell–matrix adhesion
- Loss of cell–cell adhesion
- Cytotoxic agents
- Heat shock
- Viral infection

Table 1. Morphologic differences between necrosis and apoptosis

Characteristic	Apoptosis	Necrosis
Cell size	Decreased	Increased
Cell detachment	Early, as single cell	Late, in clumps of cells
Cell membrane permeability	Normal (Trypan blue excluded)	Early uptake of Trypan blue
Cell membrane budding	An early characteristic feature	Absent
Mitochondrial morphology	Normal	Swollen, with flattened cristae
Nuclear chromatin	Condensed and fragmented	No condensation fragmentation
Formation of apoptotic bodies	Characteristic	Absent
Ultimate fate of cell	Phagocytosis	Lysis
Appearance in tissue sections	Inconspicuous, usually underestimated	Injury of cells and surrounding tissue obvious and easily quantified

- Oxidants
- Bacterial toxin
- Renal ethanol

Ischemia/hypoxia

Pharmacologic agents

- Aminoglycosides
- Amphotericin B

Receptor–ligand interactions

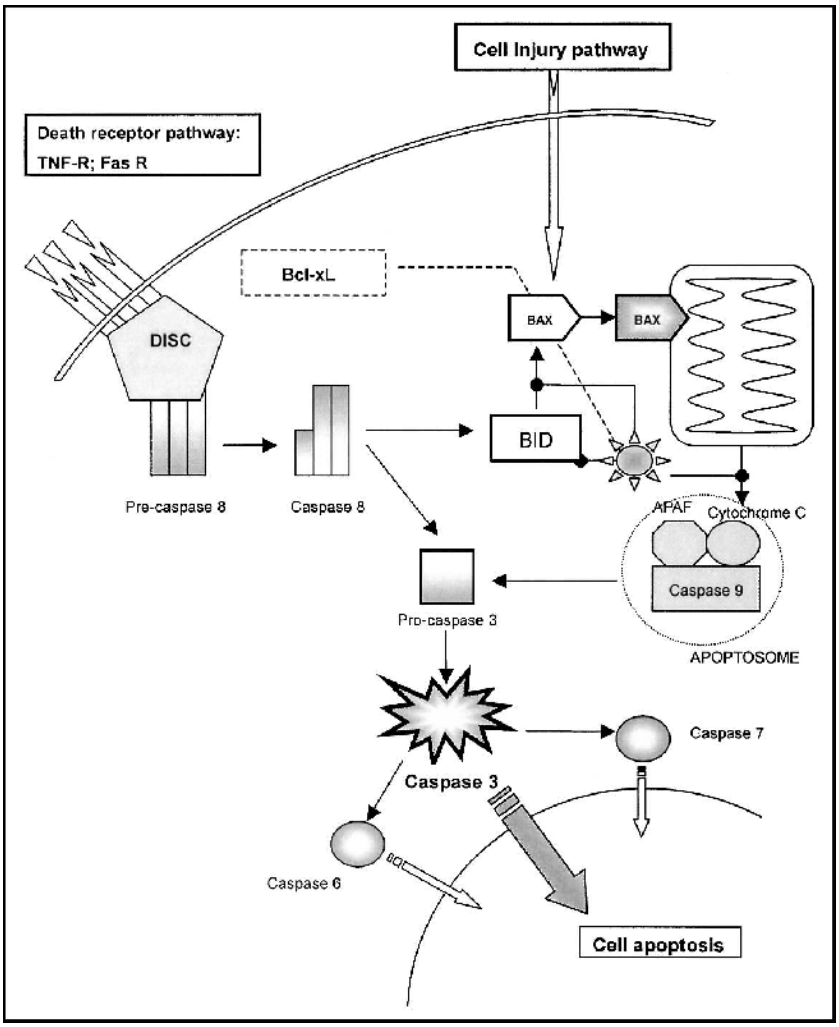
- Fas–FasL
- TNF-α–TNF receptor type 1
- Angiotensin II–angiotensin II receptor type 2

After the insult, cells undergo a cascade of intracellular events leading to apoptosis (Fig. 1).

Schumer *et al.* [23] have demonstrated that after a very brief period of ischemia (5 minutes), apoptotic bodies could be found 24 hours and 48 hours after reperfusion, and without of any evidence of necrosis. After more prolonged periods of ischemia, areas of necrosis became evident, but substantial numbers of apoptotic bodies were still seen after 24 to 48 hours of reflow. The evidence of whether apoptosis plays an important role in tubular injury *in vivo* remains controversial. It is particularly controversial whether renal cell apoptosis occurs during sepsis. However, Jo *et al.* [24•] have recently shown that apoptosis of tubular cells by inflammatory cytokines and LPS is a possible mechanism of renal dysfunction in endotoxemia. They found that if high-dose TNF was added to cultured kidney proximal tubular cells, there was increased expression of Fas mRNA, the Fas-associated death domain protein, as well as increased DNA fragmentation. Messmer *et al.* [25] have also shown that TNF and LPS elicit apoptotic cell death of cultured bovine glomerular endothelial cells, which is time and concentration dependent. Their effect was characterized by an increase in proapoptotic proteins and a decrease in antiapoptotic proteins such as Bcl-xL. Unfortunately, TNF blockade with monoclonal antibodies fails to protect animal or kidney during endotoxemia [26•]. Preliminary experiment observations by our group in septic

Figure 1. Pathways of apoptosis induced by cell injury and "death receptors"

Cell injury-induced pathway: Cell injury induces oligomerization of proapoptotic members of the Bcl-2 family of proteins, such as BAX, which translocates to the mitochondria and forms pores in the outer mitochondrial membrane that allow the release of cytochrome C (Cyto C) from the mitochondria. Cytosolic cytochrome C binds to the adaptor protein apoptotic protease activating factor-1 (APAF-1) and this complex binds to procaspase 9, forming the apoptosome, which results in autoactivation of procaspase 9. Caspase 9 activates the effector caspase 3, which activates other downstream effector caspases.



sheep also show that after only 3 hours of sepsis induced by intravenous injection of *E. coli*, there is strong expression of early-phase proapoptotic proteins such as BAX as well as counterbalancing antiapoptotic proteins such as Bcl-xL in the tubular cells, indicating that there is early activation of the apoptotic cascade in septic kidneys. Clearly, it would be attractive to have therapies that can favorably modulate the development of apoptosis.

Organ protective therapy in sepsis and acute renal failure

Activated protein C

Activated protein C (APC) is an endogenous protein that promotes fibrinolysis and inhibits thrombosis and inflammation. APC is an important modulator of the coagulation and inflammation associated with severe sepsis. During sepsis, reduced levels of APC are associated with increasing risk of death. Bernard *et al.* [27] showed a significant decrease in 28-day mortality (30.8% in the placebo group and 24.7% in the drotrecogin alfa-activated group) in 1690 septic patients treated with APC. The efficacy of APC in septic patients may be the result of its anticoagulation effect. However, a recent study by Joyce *et al.* [28] showed that recombinant human APC directly modulated patterns of endothelial cell gene expression clustering into antiinflammatory and cell survival pathways, and modulated several genes in the endothelial apoptotic pathway, including the Bcl-2 homolog protein, an inhibitor of apoptosis. More recently, Cheng *et al.* [29••] have shown that APC blocks p53-mediated apoptosis of human brain endothelium *in vitro*. APC normalized the Bax/Bcl-2 ratio and reduced caspase-3 signaling. This study creates a new link between coagulation, inflammation, apoptosis, and cell death, and provides insight into the molecular basis for the efficacy of APC in systemic inflammation and sepsis.

Caspase inhibitors

Caspases are enzymes that are believed to play a key role in apoptosis (Fig. 1). Caspase inhibitors have been developed as antiapoptotic agents. Fauvel [30] developed an animal model that showed myocardial dysfunction after endotoxin administration. He successfully used a broad-spectrum caspase inhibitor (z-VAD.fmk) and a caspase-3 inhibitor (z-DEVD.fmk), and demonstrated decreased myocardial dysfunction, reduced caspase activation, and reduced nuclear apoptosis 2 hours after experimental endotoxemia. Neviere *et al.* [31] used z-VAD.fmk 4 hours or even 14 hours after endotoxin administration in rats and showed not only that there was reduced caspase activity and nuclear apoptosis but also that endotoxin-induced myocardial dysfunction could be completely prevented. Because myocardial dysfunction can be prevented by antiapoptotic treatment, it is possible that future studies will show that the kidney is another organ that could benefit from caspase inhibitors. However, the complexity of the balance of factors involved in apoptosis and the response to sepsis is highlighted by the possibility that caspase inhibition may actually cause harm. Cauwels *et al.* [32••] have recently demonstrated that in a model of TNF-induced shock in mice, caspase inhibition was in fact associated with enhanced oxidative stress, mitochondrial damage, hyperacute hemodynamic collapse, kidney failure, and death. They found that there was a radical oxygen species-mediated pathway to lethal TNF-induced shock. Once caspase was inhibited, a caspase-dependent protective feedback on excessive radical oxygen species formation was removed, increasing lethality. These observations highlight how far we have to travel to understand the significance and complex biology of apoptosis in sepsis. Nonetheless, despite our limited understanding, some promising results have emerged from the use of an endogenous phospholipid growth factor (lysophosphatidic acid) with antiapoptotic properties. In a recent mouse model of ischemia–reperfusion [33•], lysophosphatidic acid prevented tubular cell apoptosis, loss of brush border integrity, neutrophil influx, complement activation, and loss of renal function.

Insulin and acute renal failure

The use of aggressive insulin therapy aimed at achieving euglycemia in critically ill patients has been shown to reduce mortality in critically ill patients [34]. Among the other important findings of this trial there was a dramatic reduction in the development of severe ARF requiring renal replacement therapies. A possible explanation for this finding may relate to the fact that insulin may play an important antiinflammatory role in sepsis [35•,36]. Thus, some of the beneficial effects of insulin therapy may be immune in origin rather than endocrine in nature. As such they would fit in well with the paradigm that septic ARF or ARF of critical illness may represent an immunologic/toxic state. It is also of interest that insulin has a powerful antiapoptotic effect [37] and that, conversely, high glucose concentration induces oxidative stress-mediated apoptosis in tubular epithelial cells [38•].

Low-tidal volume ventilation and acute renal failure

Ventilation of patients with the acute respiratory distress syndrome by means of a low-tidal volume strategy has been shown to reduce mortality [39]. The mechanisms for this reduction, however, remain unknown. It is possible that protective ventilator strategies might affect the well-being of other organs. In a fascinating series of studies, Imai *et al.* [40••] demonstrated that low-tidal volume ventilation might protect the kidney from injury in the setting of experimental and clinical acute respiratory distress syndrome. Using a rabbit model of acute respiratory distress syndrome, these investigators found that animals randomized to an injurious ventilator strategy had increased epithelial cell apoptosis in the kidney

as well as in the small intestine. Furthermore, such animals had evidence of renal dysfunction. When renal cells were incubated *in vitro* with plasma from rabbits exposed to an injurious ventilator strategy, apoptosis of such cells was induced and was markedly greater than that seen with exposure to control plasma. These investigators hypothesized that Fas ligand might be responsible for these changes and used FasIg (a fusion protein that blocks soluble Fas ligand) to test this hypothesis. They found that Fas ligand blockade attenuated *in vitro* apoptosis of renal cells. To confirm such an association, they obtained plasma from patients enrolled in a previous acute respiratory distress syndrome study comparing low-tidal volume ventilation with traditional tidal volume ventilation and found that there was a significant correlation between Fas ligand levels in plasma and serum creatinine.

Conclusion

Although hemodynamic factors might play a role in the pathogenesis of sepsis-induced ARF, other mechanisms are likely to be at work which are immunologic, toxic, or inflammatory in nature. Among these mechanisms, apoptosis may turn out to be dominant. Indeed, organ protective strategies recently reported in animal and human studies may work by inhibiting the development of the apoptotic cascade. It is possible that as evidence accumulates for apoptosis as a major mechanism of organ injury, the paradigm currently used to explain ARF in sepsis will shift from acute tubular necrosis to acute tubular apoptosis, and our therapeutic approach will be profoundly altered.

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