

Cell Death in Toxic Nephropathies

By Glenda C. Gobe and Zoltan H. Endre

Toxic nephropathies cause acute and chronic renal failure, primarily as a result of injury to renal tubular epithelium. There is a well-known capacity in the renal nephron for the synchronous occurrence of both apoptosis and necrosis in toxic nephropathies. This has engendered interest in the differing or complementary roles of these modes of cell death. Once thought to be mutually exclusive in incidence and morphologic and biochemical features, recent evidence in renal and other diseases indicates some blurring in the features of apoptosis and necrosis, particularly in the situations in which they are identified, in their molecular pathways, and in the role of inflammation in the processes. Definition of the heterogenic pathophysiologic response of the nephron should provide information useful for promoting the health of the kidney after injury, particularly in relation to controlling the extent and modalities of cell death via the associated renal-specific molecular features. This article indicates the significance and some problems of defining the types of cell death in toxic nephropathies.

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THE NEPHRON REACTS To toxic nephropathies with considerable heterogeneity that is still not well understood. One of the most intriguing pathologic outcomes is the concurrent death response of apoptosis and necrosis within the same nephron segments to a single injurious influence. The molecular controls that determine whether a cell dies by gene-driven apoptosis or the catastrophic loss of cellular control that causes degenerative, accidental, necrotic cell death are important to define.¹

The most common causes of toxic nephropathies are ischemia-reperfusion injury and associated hypoxia, chemical nephrotoxins (eg, drugs, heavy metals, solvents), and, in each case, toxic free radical injury. In part, the extent of injury and time for repair depend on the duration of initial injury, the type of induced cell death, and the involvement of inflammatory infiltrates. The death effectors cause acute renal failure, primarily as a result of injury to renal tubular epithelial cells. In the longer term, depending on the resolution of injury, or the continuation of tissue remodeling after repair is complete, chronic pathologies leading to chronic renal failure may develop. The temporally overlapping

occurrence of apoptosis and necrosis, particularly in the acute stage,^{2,3} has encouraged interest in their specific roles. The involvement of comorbidities such as sepsis, hypertension, diabetes mellitus, congestive heart failure, and the likelihood of progressive renal disease are equally important in patient outcome. To date, medical research has not produced data that allow a significant reduction in the high patient morbidity associated with acute renal failure after toxic nephropathies.

APOPTOSIS VERSUS NECROSIS

Although most publications refer to apoptotic and necrotic death in the tubules in toxic nephropathies, it is worth recalling that the damage within cells may be reversible. Many of the features of reversible cell injury appear to have some consistency with changes that occur in cells committed to die (Table 1).⁴ Once the commitment is made, the validity of classification of the dying cells into 2 major modes of death is supported by substantial published morphologic, biochemical, and molecular evidence. Traditionally, the circumstances in which the 2 processes were found were thought to be disparate and individually characteristic.^{4,5} Necrosis occurs accidentally after a catastrophic breakdown of regulated cellular controls that follows direct injury to the cell membrane by membrane-active toxins, or as a result of failure of the membrane pumps secondary to cellular energy depletion. Apoptosis is an active rather than degenerative process, requiring energy to proceed and being controlled genetically. Necrotic cells swell and lose membrane integrity. Apoptotic cells lose volume, maintain membrane integrity, and consequently form cellular blebs and apoptotic bodies

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Table 1. Summary of General Biochemical and Morphologic Features of Reversible Cell Injury, Necrosis, and Apoptosis

Reversible Injury	Biochemical Mechanisms	Necrosis	Apoptosis
<p>ATP depletion ATP is required for synthetic and degradative processes.</p> <p>Plasma membrane pump dysfunctional; Impaired Na⁺/K⁺ ATPase Na⁺ accumulates K⁺ moves out of cell Cells swell</p> <p>Increased osmotic load: Accumulation of catabolites, inorganic phosphate, lactate, purine nucleotides Cells swell</p> <p>Anaerobic glycolysis Glycogen stores depleted Cell pH ↓ Ribosomes detach ↓ protein synthesis</p> <p>Also Formation of cell blebs Myelin figures in plasma membrane</p> <p>Fatty change in cell cytoplasm</p>	<p>Intracellular Ca⁺⁺ ↑ Cytosolic free calcium is usually low. If membrane integrity is faulty, Ca⁺⁺ influx occurs as well as membrane release</p> <p>Enzymes activated Phospholipases (membranes) Proteases (membrane, cytoskeletal proteins) ATPases (hasten ATP depletion) Endonucleases (chromatin/DNA degradation)</p> <p>Mitochondrial damage Impaired oxidative phosphorylation, intracellular Ca⁺⁺ ↓, oxidative stress, lipid breakdown products form. Mitochondrial permeability transition (PT) pore remains closed until excessive injury. PT pore open, leakage of cytochrome c and death pathways are triggered.</p>	<p>Severe vacuolation of mitochondria</p> <p>Flocculent densities, usually Ca⁺⁺ deposits, in mitochondrial matrix</p> <p>Damage to plasma membranes</p> <p>Loss or denaturation of proteins and enzymes</p> <p>Swelling of cells and particularly the lysosomes</p> <p>Activation of lysosomal and other degradative enzymes</p> <p>Cellular degradation</p> <p>Intense acute inflammatory response</p>	<p>Condensation and margination of chromatin against the nuclear envelope</p> <p>Shrinkage of whole cell</p> <p>Blebbing of the nucleus and cell to form membrane-bound apoptotic bodies (minutes after stimulus)</p> <p>Bodies are rapidly phagocytosed by adjacent macrophages and tissue cells for degradation (within 1-2 hours)</p> <p>Little or no acute inflammatory response</p> <p>Adjacent viable cells quickly fill gaps left by dying cells</p> <p>Enzymatic cleavage at internucleosomal linker regions to form DNA sized in multiples of 180- 200 base pairs</p> <p>Changes to membranes Externalized sugar moieties, recognition and binding by phagocytes. Crosslinked protein scaffold, rigid or impermeable apoptotic cell or body membranes</p>

Abbreviations: ATP, adenosine triphosphate; PT, permeability transition.

that may or may not contain nuclear fragments. With regard to this latter characteristic, a wide variety of factors challenge constancy of cell volume. Alterations of cell volume activate diverse cell volume regulatory mechanisms including ion transport, osmolyte accumulation, metabolism, and gene expression, thus making cell volume a pathophysiologically important parameter. Necrosis usually is contiguous in nature, and after the lysis of cells there is a rapid acute inflammatory response, typically seen as an invasion of neutrophils through the viable tissue adjacent to the injury.

Apoptosis tends to occur in individual cells scattered throughout the injured area. Maintenance of the dying cell's membrane until the apoptotic cells or bodies are phagocytosed by adjacent tissue cells—an unusual phenotypic change for these tissue cells—or by invading monocytes or macrophages, means there is little acute neutrophil infiltrate, thus minimizing the secondary damage associated with an influx of acute inflammatory cells. Some of the generalized characteristics of reversible cell injury, apoptosis, and necrosis are summarized in Table 1 and photomicroscopic ex-

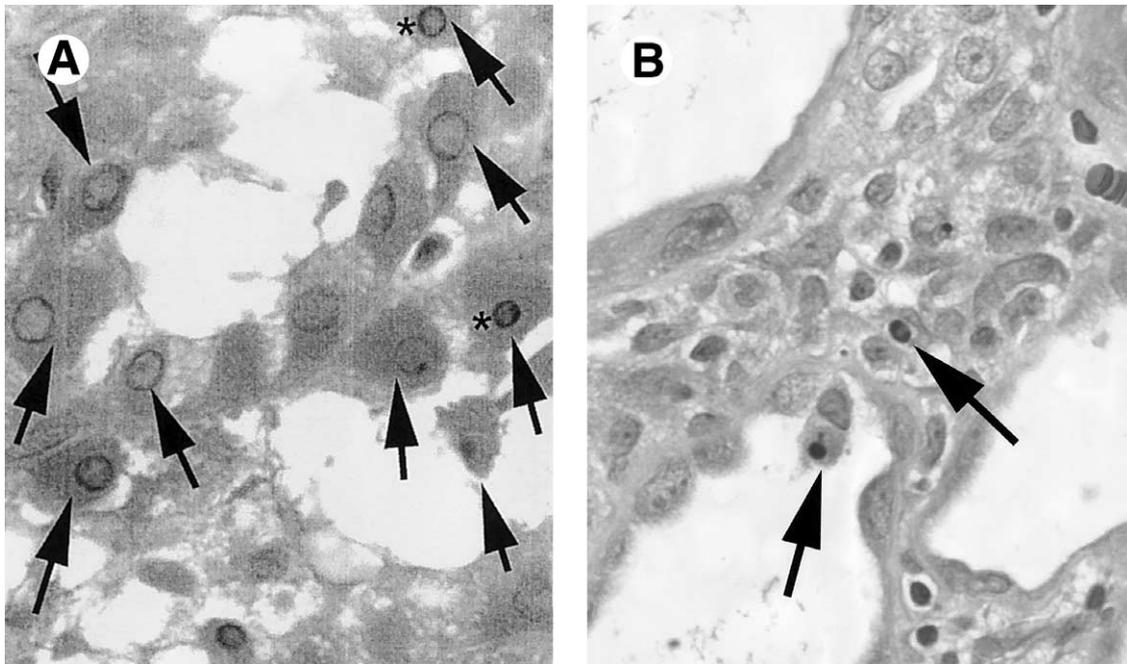


Fig 1. Morphologic evidence of necrosis and apoptosis in the kidney. (A) Necrosis (arrows) of the S3 segment of the proximal tubule 24 hours after cisplatin dosage. High amplitude cellular and nuclear swelling, fine margination (most arrowed cells), or pyknosis (*) of the nuclear DNA, cytoplasmic eosinophilia (seen as a dark cytoplasm), and desquamation of dead cells from the basement membrane are shown. Magnification $\times 480$. (B) Apoptosis in the tubular epithelium (arrows) 48 hours after ischemia-reperfusion injury. Magnification $\times 480$.

amples are given of *cis*-platinum-(II)-diammine dichloride (cisplatin)-induced necrosis (Fig 1A) and ischemia-reperfusion-induced apoptosis (Fig 1B) in the kidney.

Morphology remains a reliable means of assessing cell death although there are now several biochemical correlates, particularly for apoptosis, that may be used to verify morphologic assessment. The most common of these is in situ biochemical labeling that tags fragmented DNA associated with late-stage apoptosis with a chemical that can be visualized microscopically, or the use of immunolocalization of activated cysteine proteases, or caspases, known to act early in apoptosis. Kerr et al⁵ suggested that the use of 3 criteria, namely, morphology, biochemical verification, and expected incidence, helps assign the type of cell death being assessed when there is some doubt to its type.

APOPTOSIS VERSUS NECROSIS IN THE RENAL NEPHRON

The presence of the 2 forms of cell death in renal disease has been recognized for a little over a

quarter of a century. The cryptic and asynchronous nature of apoptosis, and preference for apoptotic renal tubular epithelial cells to bleb into the tubular lumina and be removed rapidly along with the glomerular filtrate, rather than be engulfed by adjacent phagocytic cells,⁶ meant it was some years before the significance of the apparently moderate numbers of renal cells deleted by apoptosis was given credibility. In contrast, the often-contiguous, easily identified lytic characteristics of necrosis were by far the most commonly described in renal pathologies. There has been an exponential growth in publications citing renal cell apoptosis over the past 10 years. Anoikis,⁷ or the desquamation of viable renal tubular epithelial cells, followed by their apoptosis (Fig 2), is yet another factor in the deletion of renal cells. The roles played by each mode of cell deletion have to be defined fully, but it is logical to conclude that, acutely after nephrotoxic insult, apoptosis and necrosis are a major cause of tubular cell dysfunction as a result of cell deletion, whereas in the long term, apoptosis tends to be maintained for tissue remodeling after the

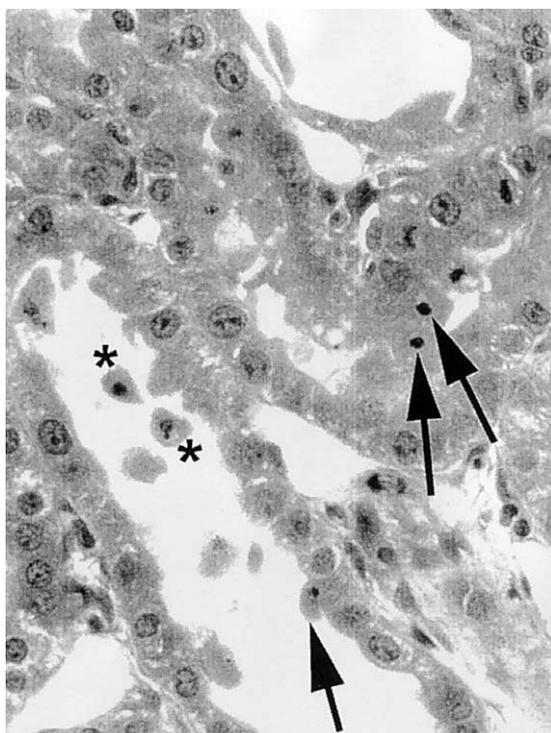


Fig 2. Anoikis (*) and apoptosis (arrows) in the tubular epithelium 48 hours after cisplatin dosage. Magnification $\times 480$.

repair phase is completed.² This latter characteristic is shown in Figures 3A and 3B, in which a healed fibrotic lesion developed 2 weeks after cisplatin-induced nephropathy. Within the fibrotic lesion, indicated in Figure 3A, apoptotic bodies and cells can be found in dilatated tubules (seen at high-power magnification in Fig 3B), leading to progressive atrophy of the tubular epithelium within the fibrotic lesion.

Susceptibility to necrotic or apoptotic injury varies along the nephron depending on the nature and duration of the injury, hemodynamic changes, ongoing metabolic demands, and local oxygen availability.⁸ For example, mercury poisoning can affect the glomeruli.⁹ Other nephrotoxins such as bismuth injure all S1, S2, and S3 segments of the proximal tubule, usually via necrosis.¹⁰ The S3 segment of the proximal tubule and the thick ascending limb of the distal nephron in the outer stripe of the outer medulla are particularly sensitive to ischemic injury, with both apoptosis and necrosis identified.⁸ Likewise, some nephrotoxic drugs such as cisplatin or metals such as cadmium

affect the same zone of the kidney but the acute damage appears to involve predominantly necrosis in the S3 segment, with some concurrent apoptosis.¹¹⁻¹³ The concentrating ability of the kidney via the countercurrent mechanism causes some nephrotoxins, particularly some analgesic drugs, to be concentrated in the tip of the renal papilla, causing necrosis in the thin limbs and collecting ducts in this zone of the kidney, and in the longer term, apoptosis and atrophy of the cortical nephron segments.^{14,15} One of the fascinating characteristics in the renal nephron is that the same insult may cause temporally overlapping apoptosis and necrosis even within the same nephron segment. Why this occurs has yet to be defined fully. Very few of the published studies have attempted to integrate information on biochemical and molecular characteristics of nephron segments, or individual nephron cells, with death outcome.

One suggestion for future study is an extrapolation from research into brain ischemia by Hou and MacManus.¹⁶ Apoptosis is known as a rapid form of cell death, executed within a few hours after initial injury and able to be visualized microscopically within 4 to 8 hours.⁵ The morphologic features of frank necrosis appear more slowly—over 24 hours. Apoptosis therefore may be the first commitment to death after injury. The final morphologic cell death features may be determined by maintenance of the process, or its abortion because of severe energy depletion. In instances in which high levels of apoptosis are found, energy for the process must be maintained, for example, for action and cleavage of energy-dependent caspases, a family of proteins central to the apoptotic process. This may occur because of a better residual blood supply or a better resistance to the effects of hypoxia or toxins. Defining the energy-related features of the renal nephron is only one means of explaining its disparate responses in cell death. Another important consideration is that there is now clear evidence that necrosis may be under some of the same molecular death controls as apoptosis,^{17,18} thus placing a question on the passive nature of necrosis and offering some means of modulating necrosis as well as this more-accepted feature of apoptosis.

ROLE OF INFLAMMATION

One of the great benefits ascribed to apoptosis versus necrosis is the general lack of inflammation

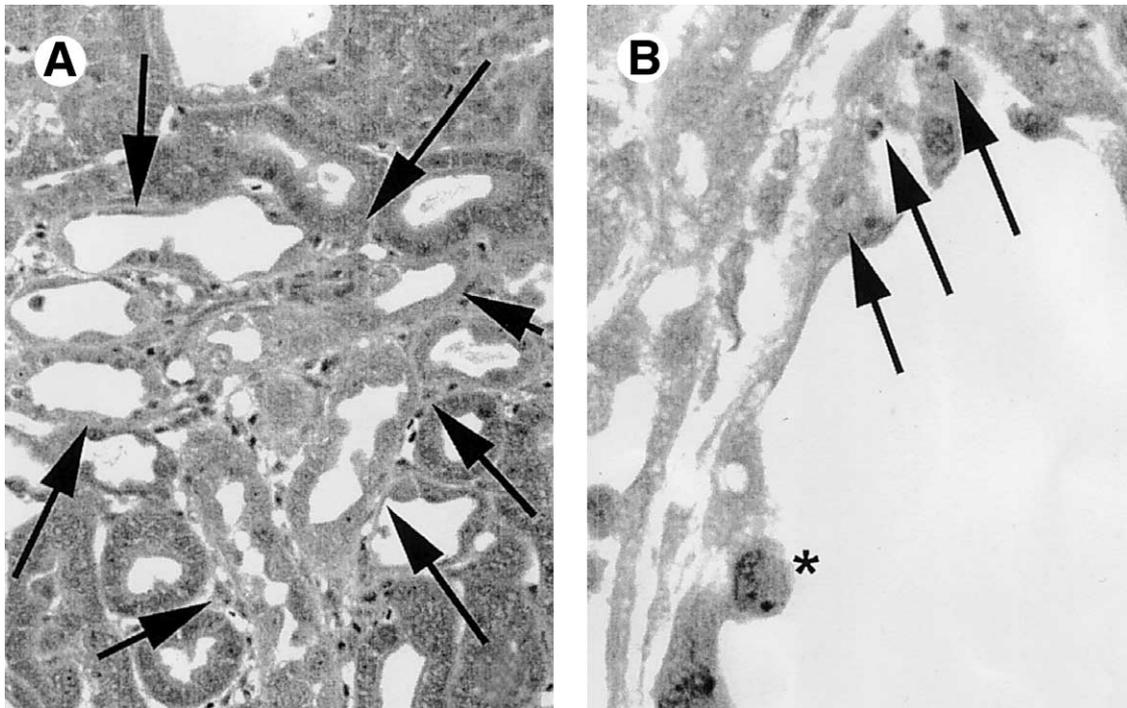


Fig 3. Chronic effects and remodeling 2 weeks after cisplatin-induced injury. (A) Arrows indicate the borders of a healed fibrotic lesion that has developed after cisplatin-induced nephropathy. (B) Within this lesion, apoptotic bodies and cells can be seen at high-power magnification within the tubular epithelium (arrows) and also blebbing into the tubular lumen (*). Magnification $\times 640$.

that occurs in the former process, making it of greater holistic benefit to an organism than the rapid and acute inflammatory response associated with necrosis. The nature of the response has important consequences for the injured kidney because inflammation in itself is damaging. The inflammatory cells, by their very phagocytic nature, contain many lytic enzymes that are expelled into the tissue on death of the inflammatory cells in situ. They produce many toxic factors, such as tumor necrosis factor α (TNF- α), as well as releasing reactive oxygen species via elevation of mitochondrial transmembrane potential.

The lineage of the initial invading inflammatory cells depends to a great extent on the cause of injury and the death type. Cell lysis of necrosis induces a massive chemotactic response that typically involves neutrophils. Necrotic cells and debris ultimately are removed by these phagocytes, which may precede monocytes, lymphocytes, and macrophages that usually invade as part of the healing and rebuilding processes. Infiltration of neutrophils around apoptotic cells traditionally has been considered minimal or absent. Monocytes and

macrophages have long been described as part of the phagocytic process of end-stage apoptosis. This may not be the full story in renal nephropathies. In the kidney, there are some published links between apoptosis and inflammation. For example, there are now several publications by Daemen et al¹⁹ that show close links between apoptosis and acute inflammatory infiltrates in the pathology of ischemia-reperfusion injury. These researchers found that minimizing apoptosis with a general inhibitor of caspases also diminished inflammation. Similarly, expression of the macrophage inflammatory protein MIP-2 and neutrophil influx in the first 24 hours after ischemia reperfusion were diminished when apoptosis of tubular epithelial cells was minimized by pan-caspase inhibition.²⁰ However, as discussed by Andrade et al¹ and Bonegio and Lieberthal,²¹ the extent to which the protective effect of pan-caspase inhibition acts via inhibition of apoptosis or, in some instances, necrosis,^{16,17} or via reduced inflammation, is not clear. There may in fact be considerable heterogeneity of response to the different (ischemic, chemical, free radical) causes of toxic nephropathies.²² From personal ex-

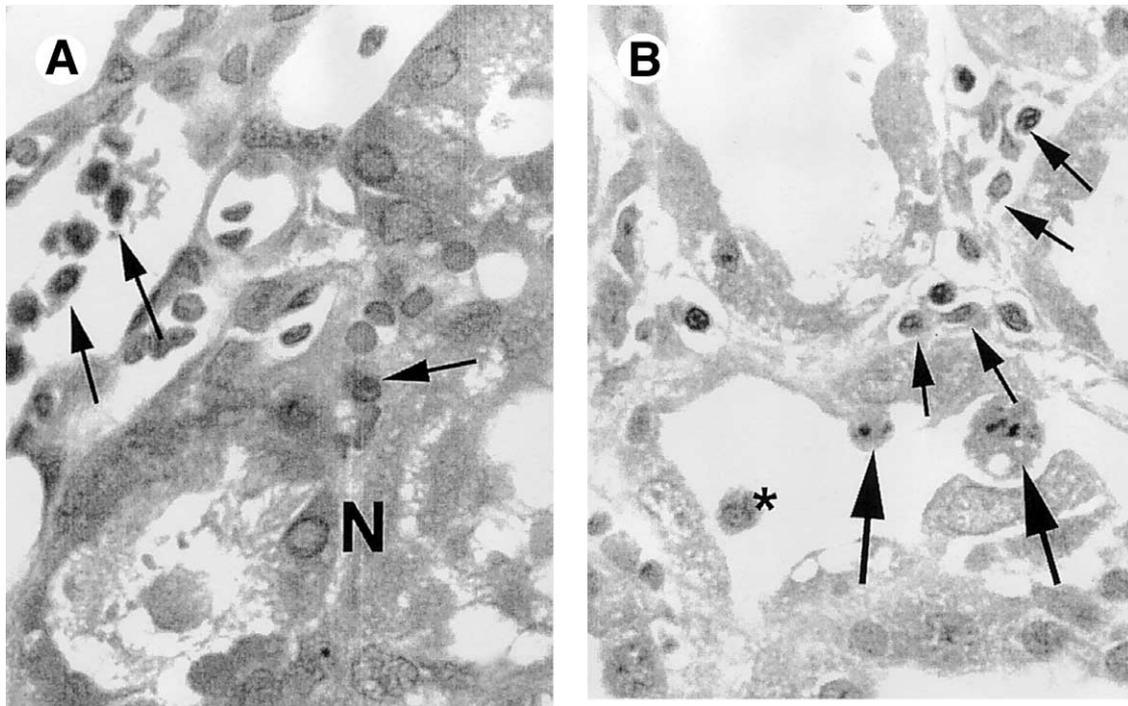


Fig 4. Acute inflammatory infiltrates in instances of necrotic and apoptotic cell death in the renal tubular epithelium. (A) Necrosis (N) after cisplatin dosage is associated with increased incidence of inflammatory cells within local vessels and the interstitium (arrows). None of the inflammatory cells was seen to have the multilobar characteristics of neutrophils, despite the quite extensive induction of necrosis. Magnification $\times 640$. (B) Similarly, apoptosis (large arrows) found in ischemia-reperfusion injury has an associated interstitial inflammatory cell infiltrate (small arrows) with characteristics of monocytes, or perhaps lymphocytes, in morphology. Note there is a cell with apparently normal DNA morphology in the tubular lumen (*), showing early induction of anoikis in this example. Magnification $\times 640$.

perience, considerable necrosis or apoptosis of the tubular epithelium may be initiated in toxic nephropathies and, provided the basement membranes of the renal nephrons remain intact, as often occurs, there is minimal neutrophil infiltration into the kidney, with the main type of inflammatory infiltrate having the morphologic characteristics of monocytes (Figs 4A and 4B).

THE MOLECULAR SCALES

Possibly the first molecular response of the renal nephron to any cytotoxic stress is activation of a variety of signal transduction pathways that culminate in cell death, particularly apoptosis, as well as other responses important in renal disease, such as proliferation, growth arrest, hypertrophy, and cell differentiation.²³ The intracellular signaling events ultimately lead to the transcription of genes whose encoded proteins mediate the response. Many of the death stimuli act via a set of cellular kinase

cascades termed collectively as the mitogen-activated protein kinase (MAPK) cascades that are made up of extracellular signal regulating protein kinase, stress-activated protein kinase, and p38 MAPK. The stereotypical view of extracellular signal regulating protein kinase acting in proliferation and stress-activated protein kinase and p38 MAPK acting in death pathways now is being questioned and this will undoubtedly continue to be a field of study that is worthwhile of investigation for defining early death pathways in toxic nephropathies.

As has been mentioned, tubular epithelial cell death is a characteristic of acute renal failure after toxic nephropathies. Although many of the molecular mechanisms are unclear, cell fate depends on a balance between the death inducers and the activity of survival and pro-death molecules. A relative deficit of survival factors in some cells of the nephron may contribute to their inability to cope

with persistent lethal factors. This is apparent when the intrinsic presence of growth factors and ability of the nephron segment for their synthesis is considered. For example, the outer stripe of the outer medulla is particularly sensitive to some toxic nephropathies. Within this zone the proximal tubules are most sensitive and often die by necrosis or desquamate. In the distal nephron, apoptosis is the more common form of cell death.^{24,25} The distal segment of the nephron (the thick ascending limb, the distal convoluted tubule, and the collecting duct) is a known reservoir of reparative growth factors for renal tissue²⁶ whereas the proximal tubule segment has receptors for the growth factors but is thought not to synthesize them. This could explain some of the disparate responses in the kidney to cytotoxic stress. Research over the past few years has shown that an intricate interrelationship may exist between growth factors synthesized in the distal tubule under the protection of up-regulated anti-apoptotic Bcl-2 proteins and distal-to-proximal cell cross-talk for protection or repair of the proximal nephron segment.^{25,27} This is discussed in more detail later.

There is now general acceptance of a dual molecular pathway initiation of apoptosis²⁸ and some indication that similar pathways act in necrosis.^{17,18} One pathway acts via the mitochondria and the other is considered to be independent of mitochondrial effect. Two major apoptosis-regulating gene families have been described—the caspases that are key proteins in cell disassembly, and the Bcl-2 gene family that is made up of both pro- and anti-apoptotic members.

The first pathway to be considered here is the mitochondrial pathway. If there is a central point at which blurring of the death processes occurs, this point may well be at the mitochondria. Mitochondrial swelling and appearance of flocculent densities, probably via increased mitochondrial calcium deposits, have long been described as the first sign of irreversible cell damage leading to necrotic death.⁴ The mitochondrial membrane potential is the driving force for mitochondrial adenosine triphosphate synthesis and it is disrupted during cell injury. The loss of function is believed to be caused in part by opening of the permeability transition pore, a mitochondrial megachannel situated at the contact point between the inner and outer mitochondrial membranes. The voltage threshold of the permeability transition pore is strongly af-

ected by oxidative stress, increasing the probability of pore opening and leakage of toxic factors such as cytochrome c into the cytosol.

Regulation of the permeability transition pore complex appears to be controlled by the Bcl-2 family of pro- and anti-apoptotic proteins for induction of apoptosis. For example, pro-apoptotic Bax can open it and anti-apoptotic Bcl-2 and Bcl-X_L are able to stabilize and inhibit its opening.²⁹ Any subsequent mitochondrial permeabilization is known to accelerate cell disassembly by amplifying caspase activity, often finally acting via activation of caspase-3.³⁰ There is now recent evidence that activation of another of the caspases, caspase-2, occurs after cytotoxic stress. This caspase is required upstream of the mitochondria for permeabilization of their membrane.³¹ As mentioned earlier, general caspase inhibitors can inhibit both ischemia-reperfusion-induced apoptosis and associated inflammation.^{19,20} It should be noted that in other tissues, rescue from apoptosis does not always prevent the cells from undergoing delayed death in the form of necrosis. Moreover, the caspase inhibitors do not always prevent the loss of transmembrane potential and release of cytochrome c, leading to what appears to be a caspase-independent death.^{32,33} With regard to the disparate death response in the renal nephron, it would be interesting to measure the levels of whole versus cleaved caspase proteins in each segment of the nephron, before and after nephrotoxic injury.

The second death pathway that acts independently of the mitochondria is often receptor activated. The caspases remain involved. In this pathway, cytokines activate caspases by assembling receptor complexes that then have a direct effect on activation of caspases, often caspase-8 or caspase-12. Activation of the TNF receptor family members such as Fas and TNF receptor-1 is the most commonly reported. In particular, the signaling cascade through which renal ischemia reperfusion induces TNF production may be central to cell death and inflammatory reactions induced. For example, oxidants released after reperfusion activate p38 MAPK and the TNF transcription factor, nuclear factor- κ B, leading to subsequent TNF synthesis.³⁴ The synthesized TNF may then loop back onto its receptors in a positive feedback manner, acting as a proinflammatory cytokine to reactivate nuclear factor- κ B. This provides a mechanism by which TNF can up-regulate its own expression as

well as facilitate the expression of other genes pivotal to the inflammatory response. After its production and release, TNF results in renal tubular epithelial cell apoptosis and dysfunction. Similar pathways must exist for other death factors acting in toxic nephropathies.

CONCLUSION

The apoptotic pathways, and possibly even the mechanisms that control induction of necrosis, offer many potential targets for modulation of cell death in renal tubular epithelial cell injury after toxic nephropathies. The description of the different modes of cell death in toxic nephropathies is important, particularly in instances in which both forms of cell death may be temporally and spatially overlapping in occurrence. Energy status and individual compensatory mechanisms in differing parts of the renal nephron need definition. The role of altered cell volume in disease is a challenge that requires more experimental research and clinical investigation. Many of the death pathways appear to converge for activation of the caspase family of proteases. There also are caspase-independent pathways limiting the use of caspase inhibitors for modulating apoptosis. Close collaboration between renal physiologists, pathologists, clinical biochemists, and molecular biologists is now needed to make the most of the information available on specific nephron responses to better define nephrotoxic injury and delineate mechanisms for modulation of cell death.

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