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Invited Review
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Contribution of apoptotic cell death to renal injury

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Abstract

Cell number abnormalities are frequent in renal diseases, and range from the hypercellularity of postinfectious glomerulonephritis to the cell depletion of chronic renal atrophy. Recent research has shown that apoptosis and its regulatory mechanisms contribute to cell number regulation in the kidney. The role of apoptosis ranges from induction to repair and progression of renal injury. Death ligands and receptors, such as TNF and FasL, proapoptotic and antiapoptotic Bcl-2 family members and caspases have all been shown to participate in apoptosis regulation in the course of renal injury. These proteins represent potential therapeutic targets, which should be further explored.

Keywords: apoptosis • kidney • acute renal failure • glomerulonephritis • Bcl-2 • death receptor • caspase

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Introduction

Cell number abnormalities in renal injury

Tissue cell number is carefully regulated through the balance between cell birth (mitosis) and cell death, with occasional participation of cell migration or cell transdifferentiation. Cell birth and cell death are intertwined and their rate frequently increases or decreases coordinately. An imbalance between these processes can result in disorders of cell number characterized by an excessive (*e.g.* neoplasia or proliferative glomerulonephritis) or insufficient cell number (*e.g.* neurodegenerative diseases or renal atrophy).

Two modes of cell death have been differentiated: apoptosis and necrosis. Apoptosis was defined morphologically in 1972, but until the late 80s its functional and therapeutic implications were not fully evident. For this reason the term necrosis in the older literature is not indicative of a specific form of cell death.

Apoptotic cell death is an active process (cell suicide) under molecular control [1-7]. Indeed, apoptosis is better defined by the requirement of energy for cell death to proceed. However, as we discuss below, the distinction between different forms of cell death is not always clear-cut. Hence, from a therapeutic point of view we are interested in any form of cell death that can be manipulated by maneuvers designed to interfere with apoptosis. Teleologically necrosis is an accidental cell death, while apoptosis is an organized dismantling of cellular structures designed to limit tissue damage. Apoptosis is an essential process to remove unwanted and harmful cells and maintain homeostasis of cell number. Most tissues, and especially the skin, gut, and immune system, depend on well-ordered apoptosis and cell replacement for normal functioning. For this reason any therapeutic measure that interferes with apoptosis should be targeted as narrowly as possible to a single cell population.

Apoptosis is characterized by morphological and functional changes. Apoptotic cells detach from the culture substrata or basal membranes and undergo cell and nuclear shrinkage, nuclear condensation, membrane blebbing and cell and nuclear fragmentation. DNAses fragment DNA in internucleosomal sized fragments. Cell membrane integrity is preserved until advanced stages of the process. However, the composition of the cell membrane changes, allowing the rapid recognition and engulfment of apoptotic cells by healthy adjacent cells, before they lose cell membrane integrity and leak proinflammatory molecules. As a result the half-life of the apoptotic morphology is short (1-2h) and apoptosis does not generate florid inflammation. Both factors make apoptosis inconspicuous. Even in tissues suffering a significant cell loss through apoptosis, the amount of visible apoptotic cells remains low.

The classic example of necrosis is ischemic cell death, characterized by an increase in cell volume and an early loss of cell membrane integrity.

Differences between apoptosis and necrosis are not absolute, and the term necrapoptosis has been coined. Cells suffering potentially lethal metabolic changes often try to commit suicide by activating apoptosis pathways before they are killed. However, inactivating these apoptosis pathways may not save the cell from death. The intensity of the lethal stimulus, the availability of ATP (energy) or the inactivation of caspases may determine whether the cell dies through apoptosis or necrosis [6,8]. Moreover, apoptotic cells that are not engulfed by adjacent cells undergo secondary necrosis defined as a loss of cell membrane integrity. Finally, survival proteins such as Bcl-2 and Bcl-x_L protect against cell death with morphology of either apoptosis or necrosis [5].

Apoptosis is tightly regulated by extracellular and intracellular molecules that provide multiple regulatory and contraregulatory pathways. We will review the current understanding of apoptosis regulation, making reference to renal cells when such data are available.

The extracellular microenvironment

Cell death is usually a response to the cell microenvironment, where the absence of certain factors (survival factors) or the presence of lethal factors promotes apoptosis. Surrounding cells, soluble mediators and the extracellular matrix regulate cell death and survival. Surrounding cells can synthesize survival or lethal factors or compete for such factors.

Survival factors

Most cells need survival signals from their surroundings to remain alive. However the survival factors vary depending on cell type and functional status. Survival factors for human and rat mesangial cells include IGF-1, IGF-II and bFGF, while EGF, PDGF and TGFβ1 had no effect [9]. By contrast EGF, HB-EGF, HGF are survival factors for tubular epithelial cells. Collagen IV and laminin, components of normal mesangial extracellular matrix (ECM), protected rat mesangial cells from apoptosis induced by serum starvation and DNA damage, by a β1-integrin-mediated mechanism [10]. By contrast, pathological components of the glomerular ECM, such as collagen I and fibronectin offered no protection [10].

Lethal factors

Lethal cytokines belonging to the TNF superfamily bind to and activate cell membrane death receptors [4]. A fine regulation of the system protects innocent bystanders from accidental death, and includes the existence of soluble receptors and decoy receptors that behave as cytokine antagonists [4]. Moreover some lethal cytokines, such as TNF and Fas ligand (FasL), may be released from the cell membrane by the action of enzymes. The lethality of soluble FasL is up to 1000 less than that of membrane-bound FasL, and soluble FasL may even antagonize the lethal effect of membrane-bound FasL [4].

TNF and FasL have been extensively investigated in renal cells. In the kidney both cytokines may be synthesized by infiltrating leukocytes and intrinsic renal cells, the main source of intrinsic FasL being the tubular epithelial cell [11-13]. TNF and FasL can induce apoptosis of mesangial cells, tubular epithelial cells, renal endothelial cells and renal fibroblasts [11-17]. However the sensitivity of these cell types to cell death varies with the cell microenvironment. Under basal conditions tubular cells are quite resistant to FasL-induced apoptosis, as expected by their constitutive expression of the cytokine. However, they become sensitized to FasL lethality upon exposure to inflammatory cytokines [13-15]. By contrast Fas activation induces death in non-stimulated mesangial cells *in vitro* and *in vivo* and Fas-induced death is increased by inflammatory cytokines [16-18]. TRAIL has not been shown to be lethal to renal cells.

Lethal factors that cause receptor-independent cell stress usually kill by a mechanism involving mitochondria. Cellular stress can induce the expression of death ligands and receptors. However, their inhibition or antagonism may not rescue the cell from death. Lethal stimuli for renal cells include several nephrotoxins [1].

Interaction of survival and lethal factors

The cell microenvironment usually contains multiple survival and lethal factors. The potential for interaction between survival and lethal factors varies in a stimulus- and cell-specific manner. The absence of survival factors can predispose renal cells to death induced by lethal cytokines and nephrotoxic drugs. Other lethal stimuli, such as HMGCoA inhibitors, induce apoptosis only in actively proliferating mesangial or smooth muscle cells and spare quiescent cells grown in serum-free conditions [19]. This property could be used therapeutically to target proliferating mesangial cells *in vivo*.

Intracellular regulation of apoptosis

The intracellular regulation of apoptosis is a fast advancing field. Although different pieces of the puzzle have emerged, the exact relationship between them is open to interpretation and the schemes and pathways described in this review are, necessarily, oversimplified and subject to modifications. The basic components of the intracellular regulation of apoptosis are highly conserved in the philogenetic scale. Seminal studies in the nematode *C. elegans* defined a basic role for CED-3, CED-4, CED-9 and EGL-1 proteins in apoptosis (Fig. 1)[2]. A comparison of the proteins encoded in the human genome to those from the fly and nematode genomes reveals a major increase in the complexity of the apoptotic molecular machinery in vertebrates, in terms of both the number of proteins involved and their domain architecture [20].

The cell death process is activated by intracellular factors in response to a lethal cell microenvironment. Known intracellular activators of apoptosis include death receptors, proapoptotic members of the Bcl-2 family, ER stress and mitochondrial injury (Fig. 2).

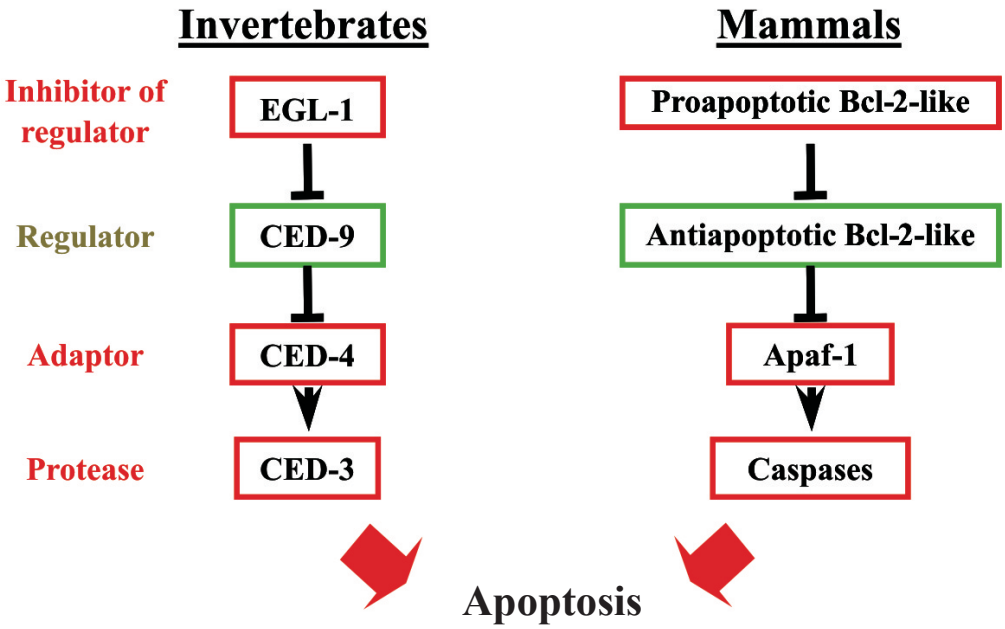

Fig. 1 Intracellular regulation of apoptosis in invertebrates compared to mammals.

Death receptors

Death receptors are members of the TNF receptor (TNFR) superfamily, which contain a cytoplasmic death domain (DD) [4]. Binding to the lethal cytokine promotes death receptor oligomerization and recruitment of several adaptor proteins and procaspases to a receptor complex (DISC: death-inducing signaling complex). The Fas receptor DISC includes FADD, an adaptor protein that contains a DD that allows the interaction with the DD in death receptors, and a death effector domain (DED) that allows it to interact with DED domain-containing procaspase-8 [4]. Procaspase-8 is cleaved and activated in the course of Fas engagement. Ligand-independent activation of cell death signaling by death receptors has been described, although not yet in renal cells. Mechanisms exist to limit the spontaneous signaling from DD-containing receptors, such as the inhibitory protein silencer of DD (SODD) and FLIP, that inhibits caspase-8 activation by binding to FADD [4].

Type I and type II are differentiated according to the efficiency in forming a death signaling complex upon FasL binding to Fas [4]. Type II cells have low efficiency in forming this complex and the lethal signal must be amplified through recruitment of the mitochondrial pathway for apoptosis. The amount of cell surface Fas is one of the determinants of cell sensitivity to Fas-induced death.

Mesangial cells, tubular cells and renal fibroblasts express cell surface Fas receptors [17]. A number of proapoptotic situations relevant to the pathogenesis of renal injury upregulate Fas expression in renal cells and, at least some of them, render the cells more susceptible to FasL-induced apoptosis. They include cytokines (TNF, IFNγ, IL-1ß, IL-1α), bacterial lipopolysaccharide (LPS), nephrotoxins, HIV infection and deprivation of survival factors [11,13-15,17].

Proapoptotic Bcl-2-like proteins

Some members of the Bcl-2-family can trigger apoptosis (Table 1). For example, Bax overexpression induces caspase-independent cell death [5]. Mechanisms for their pro-apoptotic activity include: 1) binding and inhibition of Bcl-2 or Bcl-x_L, thus triggering the release of caspases and 2) inducing the opening of mitochondrial membrane channels, thus promoting the release of mitochondrial apoptogenic factors into the cytoplasm. Bax shuttles from its cytoplasmic location to the mitochondria upon

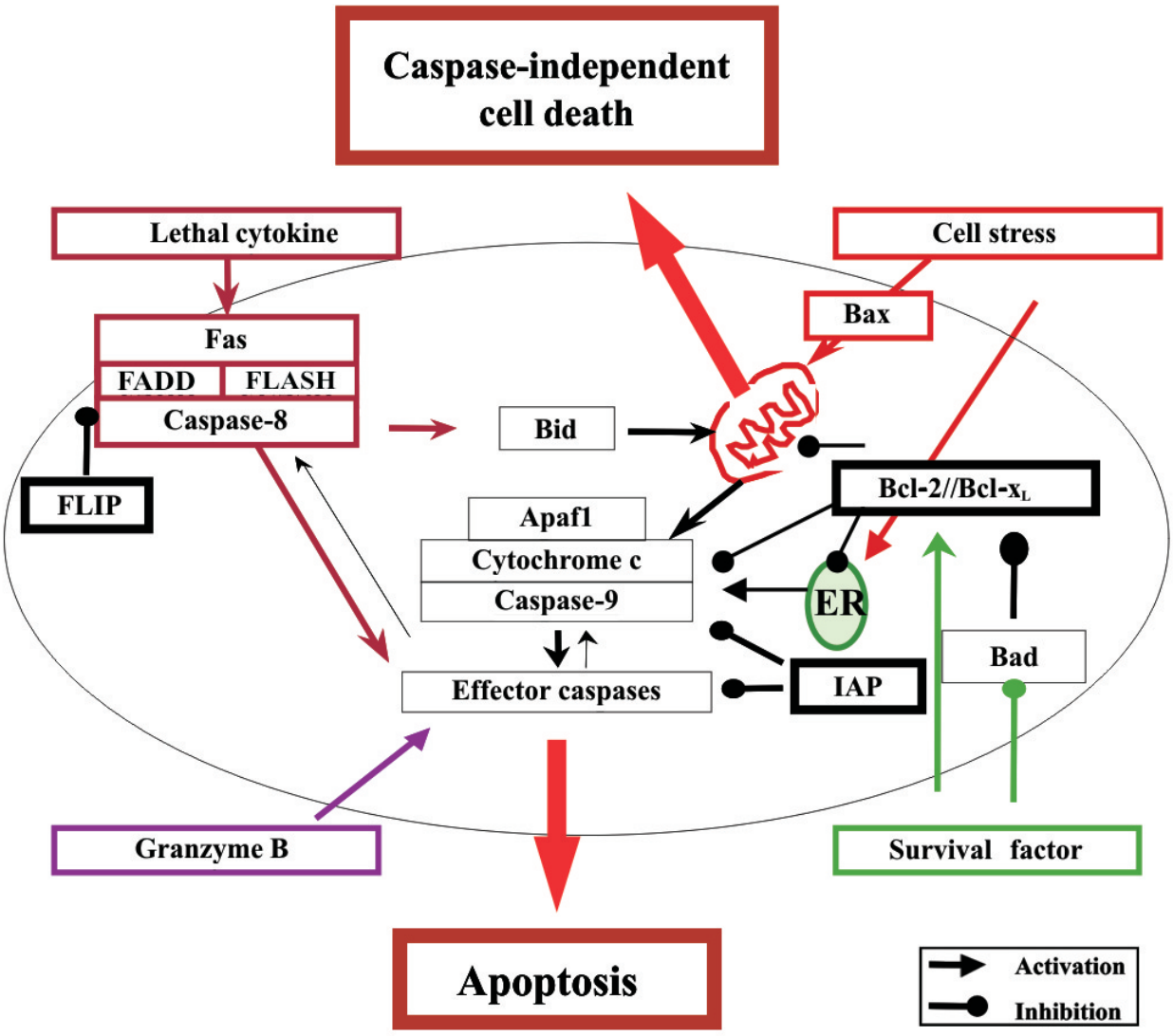

Fig. 2 Intracellular pathways for cell death. Death receptors, cell stress or granzyme B can activate cell death pathways, and extracellular survival factor promote the activity of intracellular antiapoptotic factors. Mitochondrial injury can result in caspase-independent cell death without features of apoptosis.

induction of apoptosis by glucose-free hypoxia in tubular cells while other proapoptotic stimuli, such as serum deprivation or NO, increase Bax expression in tubular and mesangial cells, respectively [21-23].

A subfamily of proapoptotic Bcl-2-like proteins containing only the BH3 domain (BH3-only proteins) has recently been characterized (Table 1).

Mitochondria

Mitochondria are key participants in apoptosis that is not triggered by death receptors, and may also contribute to cell death after death receptor activation [7]. Mitochondrial changes during apoptosis include 1) dissipation of the mitochondrial transmembrane potential gradient ($\Delta\Psi m$) due to the opening of a large conductance channel known as the permeability transition (PT) pore and 2) release of proteins, such as cytochrome c, AIF and SMAC/Diablo, from the mitochondrial intermembrane space to the cytosol, where they participate in the effector phase of apoptosis by directly activating caspases [7]. Dissipation of the mitochondrial transmembrane potential gradient and the release of apoptogenic factors can occur independently of each other. In this regard, mitochondrial injury can cause caspase-independent cell death that may not have the apoptotic morphology [7].

Table 1 Bcl-2-family proteins and their role in apoptosis

Survival proteins	Proapoptotic proteins	
	Bax subfamily	BH3-only subfamily
Bcl-2*	Bax	Bid
Bcl-x_L*, **	Bak	Bad
Bcl-w	Bok/Mtd	Bik/Nbk/Blk
Bcl-B	Bcl-x_S**	Bim/Bod
Boo/Diva		Hrk/DP5
A1/Bfl-1		Noxa
NR13		Bcl-G
Mcl1		BNIP3/NIP3
		NIX/BNIP3L

*Caspase-generated Bcl-2 and Bcl-x_L fragments may promote cell death

** Bcl-x_L and Bcl-x_S are isoforms derived from alternative splicing of the same gene

The nature of the PT pore is currently under intense investigation. There is evidence for the participation of VDAC (porin or voltage dependent anion channel) and ANT (adenine nucleotide traslocator). Cyclosporin A inhibits ANT and protects against certain modes of apoptosis.

Endoplasmic reticulum

Stress in the endoplasmic reticulum (ER) can also result in apoptosis. ER proteins that modulate apoptosis include p28 BAP31, which acts as a bridge between Bcl-2 and caspase-8 [24] Evidence is accumulating for a Bcl-2-dependent cross-talk between mitochondria and ER in the regulation of apoptosis [25]. Caspase-12 is localized to the ER and activated by ER stress, including disruption of ER calcium homeostasis and accumulation of excess proteins in ER, but not by membrane- or mitochondrial-targeted apoptotic signals [26]. Mice that are deficient in caspase-12 are resistant to ER stress-induced apoptosis, including renal injury [26].

Proteolytic enzymes

Caspases are the most widely studied apoptotic proteases. Most caspases are constitutively expressed as inactive proenzymes (procaspases) in the cytosol, and according to some reports, in the mitochondria. Caspases are sequentially activated by proteolysis during apoptosis: initiator caspases activate downstream, effector caspases (Table 2) [6]. Alternatively, proteases, such as granzyme B, that are introduced into the cell by perforin-expressing cytotoxic lymphocytes activate caspases.

Procaspases possess considerably less activity than mature caspases. As a result, recruitment and oligomerization of initiator procaspases mediated by adaptor proteins (FLASH, Apaf-1) constitutes a basic mechanism of caspase activation by proteolysis [6]. Caspase-9 is the initiator caspase of the mitochondrial pathway for apoptosis. It is activated after binding to the cytosolic adaptor protein Apaf-1. Apaf-1 itself must be activated through a conformational change that occurs in the presence of dATP when cytochrome c is released from the mitochondria [6]. Activated Apaf-1 molecules oligomerize and, together with caspase-9, form a protein complex dubbed the apoptosome. Caspases-8 and -10 are the initiator caspases of lethal receptor-induced apoptosis. They become activated upon recruitment to the DISC. Caspase-8 cleaves the proapoptotic member of the Bcl-2 family Bid and Bid fragments migrate to the mitochondria, where they promote the mitochondrial pathway of apoptosis in type II cells [4].

Initiator caspases (-8,-9,-10) activate effector caspases (-3,-6,-7). Effector caspases can, in turn, activate initiator caspases [6]. Over 40 substrates for caspases have been identified whose cleavage

Table 2 Mammalian caspases

Caspases	Function
Caspase-1 (Interleukin-1ß-convertase: ICE)	Inflammation
Caspase-4 (ICE-rel II, TX, ICH-2)	Inflammation?
Caspase-5 (ICE-rel III, TY)	Inflammation?
Caspase-2 (ICH-1)	Effector of apoptosis
Caspase-3 (CPP32, apopain, yama)	Effector of apoptosis
Caspase-7 (Mch3, ICE-LAP3, CMH-1)	Effector of apoptosis
Caspase-6 (Mch2)	Effector of apoptosis
Caspase-8 (FLICE/MACH/Mch5)	Initiation/Signaling of apoptosis
Caspase-9 (ICE-LAP6, Mch6)	Initiation/Signaling of apoptosis
Caspase-10 (Mch4, FLICE-2)	Initiation/Signaling of apoptosis
Caspase-11 (mICH-3)	Inflammation/apoptosis
Caspase-12 (mICH4)	ER apoptosis
Caspase-13 (ERICE)	Apoptosis?
Caspase-14 (MICE)	Keratinocyte differentiation

can be either an activating or inactivating event for the function of the protein. Most proteins whose cleavage leads to the characteristic apoptotic morphology are targeted by the effector caspases. Targets include inactivation of protective proteins, such as Bcl-x_L and Bcl-2, that may even yield proapoptotic fragments, dismantling of structural proteins and activation of DNAses [6].

There are natural and synthetic inhibitors of caspases. Endogenous caspase inhibitors in mammals include the caspase-8 inhibitor FLIP, the caspase-3 and caspase-9 inhibitors IAP (XIAP, cIAP1, cIAP2), and truncated, dominant-negative isoforms of caspases [6].

Inhibition of caspases can prevent cell death. This is especially true in death induced by ligation of death receptors, such as Fas, where the commitment to die depends on caspases. Thus inhibition of caspase-8 by zVAD prevents lethal receptor initiated downstream intracellular events and cell death. However, in some biological systems of mitochondrial injury caspase inhibitors prevent features of apoptosis such as nuclear pyknosis and internucleosomal DNA degradation, but do not increase overall cell survival. Experiments should be prolonged in time in order to exclude this possibility.

The complex phenotypes of the caspase knockout mice indicate that multiple mechanisms of caspase activation operate in parallel and that death signal transduction pathways are both cell-type and stimulus specific. There are at least four different death pathways, attending to the role of caspases-3 and -9 [6]. There is yet not enough information on which caspases are important in mediating renal cell death. However, caspase-12 deficient mice are protected against certain nephrotoxic agents [26].

Other proteolytic enzymes also become activated and participate in apoptosis, such as calpaine and cathepsins. m-calpain may be responsible for cleaving procaspase-12 and Bcl-x_L [27].

Protective proteins

Survival Bcl-2-like proteins (Table 2) protect from cell death in which the mitochondrial pathways for apoptosis are activated [5]. They may fail to protect from receptor induced apoptosis, one exception being type II cells, in which recruitment of procaspase-8 is inefficient and the lethal signal needs to be amplified through the mitochondrial pathway.

Proapoptotic and antiapoptotic members of the family can interact, and the overall effect on cell

survival may depend on the balance between the activity of proapoptotic (such as Bax and Bcl-x_S) and antiapoptotic (such as Bcl-2 and Bcl-x_L) proteins [5]. Thus it is difficult to draw conclusions from descriptive papers in which the expression of just one of these factors is reported. Scenarios in which antiapoptotic proteins bind to and inactivate proapoptotic proteins and in which it is the proapoptotic proteins that inactivate antiapoptotic members of the family have been described.

The mechanisms of the protection afforded by Bcl-2 family members is still been debated. Two theories have emerged. In one apoptosis is prevented by sequestering procaspases in the apoptosome. In this sense, Bcl-x_L binds to the complex formed by caspase-9, Apaf-1 and cytochrome c and prevents caspase activation [5]. A second scenario involves closing the VDAC and preventing the release of mitochondrial apoptogenic factors such as cytochrome c and AIF into the cytoplasm [5]. This effect may be related to the ability of these proteins to form transmembrane channels [5].

The survival factors in serum upregulate Bcl-2 and Bcl-x_L expression and downregulate Bax in murine tubular epithelial cells, while lethal factors such as TNF and acetaminophen downregulate Bcl-x_L [23]. High glucose concentrations, similar to those found in diabetes, upregulated bax mRNA and downregulated Bcl-2 and Bcl-x_L mRNAs in tubular epithelial cells [28]. Although neither survival cytokines nor ECM modified these proteins in mesangial cells [9,10], a NO donor and oxygen radicals upregulated Bax expression in mesangial cells [22]. Overexpression of Bcl-2 or Bcl-x_L also protected tubular epithelial cells from apoptosis induced by nephrotoxins, lethal cytokines or hypoxia-reoxygenation [23].

There are other protective proteins, such as heat shock proteins, whose antiapoptotic activity is less well characterized.

Signal transduction pathways and transcription factors

Several intracellular mediators have been implicated in death signaling. They include oxygen radicals, ceramide, and posttranslational modification of proteins such as phosphorylation, nitrosylation and proteolysis [1-3]. Furthermore, in the course of apoptosis the composition of the intracellular milieu changes and these changes facilitate caspase activation.

Some apoptosis pathways require new gene transcription (e.g. dexamethasone induced death of lymphocytes) while others do not (*e.g.* Fas induced apoptosis). In mesangial and tubular epithelial cells protein synthesis inhibitors induce apoptosis and also sensitize to apoptosis mediated by the death receptors TNFR and Fas, suggesting that ongoing synthesis of protective proteins is required to prevent cell death [16,17]. Transcription factors participate in some forms of apoptosis. However, their exact role varies with cell type and the functional status of the cell. c-myc promotes apoptosis when the concentration of extracellular survival factors is low, but favors cell division in the presence of survival factors. p53 promotes apoptosis through pathways that can be both independent and dependent on the transcriptional downregulation of Bcl-2 and upregulation of Bax. NFκB is activated upon engagement of some death receptors. One function of NFκB is to prevent cell death, as it is the case in mesangial cells exposed to TNF [29].

Molecular mechanisms linking mitosis and cell death

A number of molecular links between cell birth and cell death have been unraveled. Extracellular factors (EGF, IGF-1, HGF) have both survival and growth factor activity. Transcription factors such as c-myc, that are activated in stressed cells, regulate cell division or cell death. Some cyclins and cyclin-dependent kinases regulate both the cell cycle and apoptosis. Caspases degrade the cdk2 inhibitors p21Cip1/Waf1 and p27Kip1 and the resultant increment in cdk2 activity has been implicated in apoptosis. Overexpression of Bcl-2 or Bcl-x_L protects from apoptosis and also decreases cell proliferation.

Cell loss through apoptosis in renal injury

Cell death through apoptosis has been documented in the course of renal injury both in animal models and clinical kidney diseases, including glomerulonephritis, acute and chronic renal failure, diabetic nephropathy and polycystic kidney disease [1](Fig. 3).

Table 3 Different roles of apoptosis in renal injury

Cell type	Promotion of tissue homeostasis	Promotion of disease
Intrinsic renal cells: - mesangial cells - glomerular epithelial cells - tubular epithelial cells - vascular cells	Tissue remodeling: - kidney development Resolution of hypercellularity: - proliferative glomerulonephritis - recovery from acute renal failure	Promote/initiate renal injury: - glomerulonephritis - acute renal failure Progression of renal injury: - chronic renal atrophy
Renal fibroblasts	Clearance of interstitial fibroblasts	
Leukocytes	Clearance of inflammatory cells	
Lymphocytes	Limitation of the immune response	
Any		Inflammatory cell recruitment?* Autoimmunity: apoptosis neoautoantigens*

* These mechanisms have been invoked when apoptotic cell phagocytosis fails

Role of apoptosis

Both apoptosis of intrinsic renal cells and of infiltrating leukocytes may contribute to the pathogenesis of renal disease (Table 3). Apoptosis participates in the loss of parenchymal cells at several stages of renal injury. Apoptosis of intrinsic renal cells may be deleterious or beneficial and its role may change in the course of renal injury. There is evidence suggesting that apoptosis can be a cause of renal damage, a mode of restoring normal tissue structure and a mechanism for persistence and progression of renal injury.

Apoptosis triggered by ischemia, exogenous toxins or endogenous mediators of damage may be the **initial insult** that causes renal disease. Both agonistic anti-Fas antibodies and anti-Thy-1 antibodies induce mesangial cell apoptosis *in vitro* and *in vivo* [16,18,30]. Agonistic anti-Fas antibodies cause complement-independent, acute, mesangial cell apoptosis, that is associated with transient proteinuria and hematuria [18]. By contrast anti-Thy-1 antibodies cause complement-dependent mesangial cell apoptosis that is followed by self-limited proliferative glomerulonephritis [30]. Tubular cell death in the early stages of ARF can proceed through apoptosis or necrosis [31,32]. The relative contribution of the two mechanisms to the initial tubular cell loss is uncertain, and may depend on the severity of the insult.

Apoptosis also contributes to **tissue remodeling and recovery of normal tissue structure**. A physiological example is kidney development. Apoptosis decreases the mass of unneeded metanephric mesenchyme following induction by the ureteric bud and altered apoptosis during kidney development can result in renal dysplasia or agenesis. Evidence from experimental models of renal injury suggests that apoptosis coexists with renal cell proliferation. In this case apoptosis represents a physiologic balance to clear redundant cells and resolve an exaggerated compensatory proliferative response to injury, such as proliferative glomerulonephritis or tubular hyperplasia in the recovery phase of ARF [31]. Insufficient fibroblast apoptosis can promote accumulation of interstitial fibroblasts during renal scarring, as fibroblasts involved in wound repair are eliminated by apoptosis.

When a high apoptotic rate, relative to the mitotic rate, of renal parenchymal cells persists beyond the recovery of normal tissue cellularity, it leads to the loss of renal parenchymal cells that characterizes **progression of glomerular sclerosis or tubular atrophy**. Understanding the molecular basis for the continued cell loss is of utmost importance. The contribution of persistent apoptosis to mesangial and endothelial cell depletion has been documented in experimental models of progressive glomerular scarring, such as crescentic anti-glomerular basement

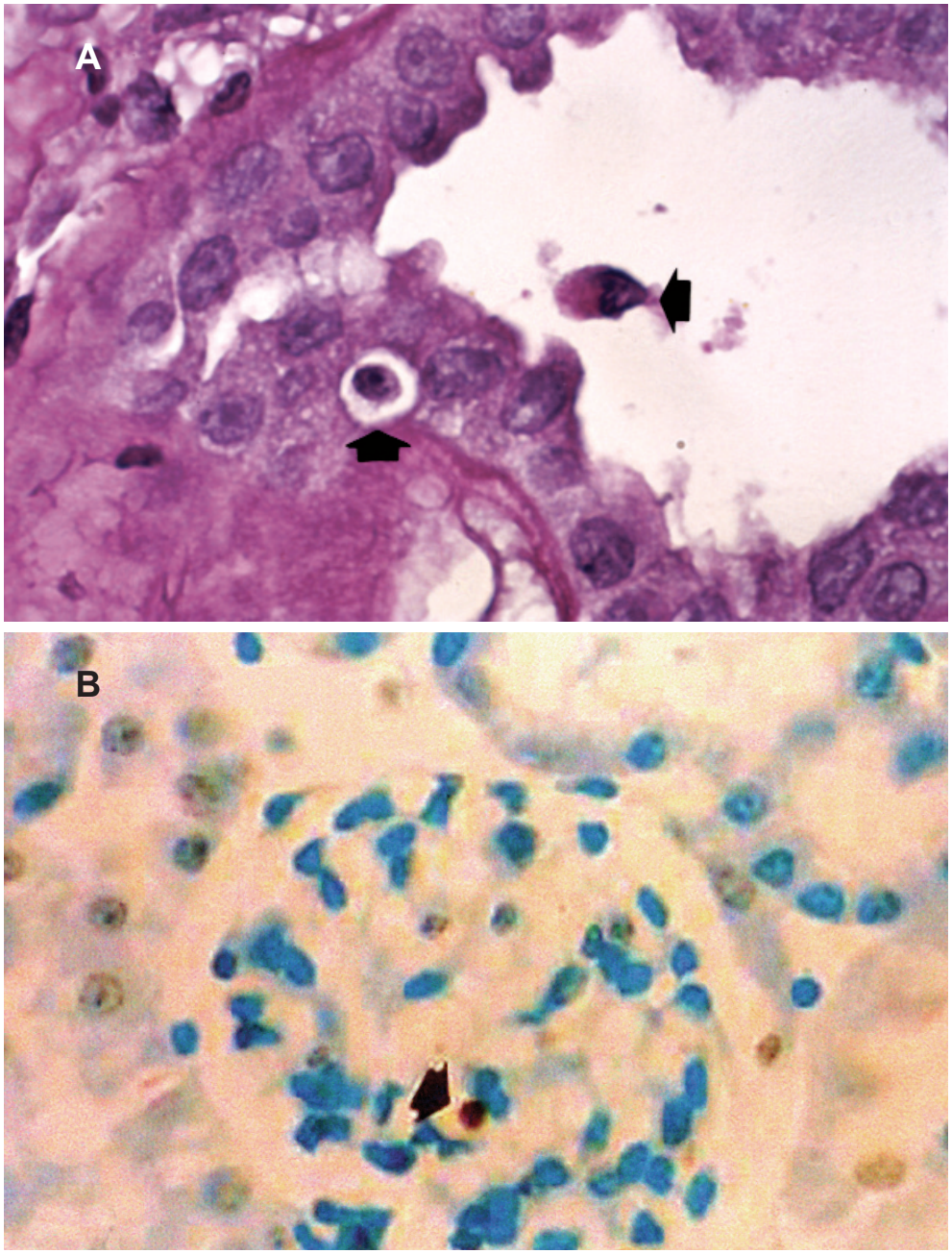

Fig. 3 **A**. Apoptotic tubular cells in the tubule wall and lumen in clinical acute renal failure (arrows)(PAS, original magnification x100). **B**. Glomerular cell apoptosis (arrow) in the course of experimental anti-Fas induced glomerular injury (TUNEL, original magnification x250).

membrane antibody-induced nephropathy [33]. An increased rate of apoptosis was observed in human proliferative nephritis such as IgA nephropathy and lupus nephritis, especially in sclerosing lesions. Follow-up studies are missing in human biopsies, so any conclusion on the consequences of apoptosis in the clinical setting has to be based on previous experimental data. Indeed, either leukocytes or intrinsic renal cells may be undergoing apoptosis. If leukocytes are being cleared, then apoptosis might contribute to healing. Moreover, mesangial cell apoptosis may keep in check an exaggerated proliferative response, although if too vigorous, leads to glomerular cell depletion. An excess rate of apoptosis of tubular epithelial cells has been observed in experimental models of chronic tubular

atrophy and proteinuric glomerular injury and human HIV nephropathy [34-36].

Apoptosis also **regulates inflammation.** Clearance of inflammatory cells by apoptosis contributes to resolution of renal inflammation There is a harbored notion that apoptosis does not generate inflammation. Nevertheless, the relationship between apoptosis and inflammation should be reevalauted, as a mononuclear cell infiltrate frequently accompanies sites of apoptosis. Apoptosis itself might promote inflammation through two mechanisms: 1) Disintegration of apoptotic cells with release of non-specific pro-inflammatory factors may be a consequence of a failure of the recognition/engulfing mechanism. 2) Active release of proinflammatory cytokines. In fact, inhibition of apoptosis resulted in decreased renal inflammation following ischemia-reperfusion [37].

Apoptosis of lymphocytes plays a fundamental role in the control of the immune response in the thymus and the periphery. Altered expression of apoptosis-related proteins such as Bcl-2, Fas and FasL results in autoimmunity and renal damage [12]. Apoptosis by itself may generate autoimmunity, as autoreactivity has been recognized against antigens present in apoptotic cells. If apoptotic cells are not adequately cleared, their contents might be released and further stimulate this autoimmune response.

Regulation of apoptosis

The characterization of the factors that regulate apoptosis during renal injury in vivo has lagged behind the identification of apoptosis as a participant in renal cell homeostasis. This lack of information, which is specially noteworthy in humans, has hindered the development of therapeutic strategies based on modulation of apoptotic cell death. Piecemeal data have emerged regarding the expression of some of the earliest recognized apoptosis regulatory proteins during glomerulonephritis, tubular injury and polycystic kidney disease (PKD) [1].

Changes in the expression of apoptosis regulatory factors have been observed in proliferative **glomerular injury** rather than in non-proliferative glomerulopathies. The interpretation of these studies is frequently hampered by the difficulty for differentiating the cell type (intrinsic glomerular cell vs leukocytes) that is expressing the apoptosis regulatory protein, and, in human material, by the lack of follow-up studies. Furthermore there are few functional studies that address the role of specific apoptosis regulatory proteins in modifying the evolution of glomerular injury *in vivo*. Expression of lethal factors such as TNF and FasL is increased in proliferative glomerular injury [13]. Increased expression of Fas, Bcl-2 and Bax has been observed within the glomerulus in human proliferative glomerulonephritis. However, Bcl-2 positivity was limited to less than 2 cells/glomerulus and it could represent expression by infiltrating leukocytes.

The expression of both extracellular and intracellular apoptosis regulatory factors changes during **ARF**. A cytokine microenvironment permissive for cell death includes decreased renal levels of survival factors and increased cell membrane survival factor receptors, indicating competition for survival factors. Increased TNF and FasL have also been observed in ARF and are matched by high renal Fas expression in experimental models of ARF. Furthermore, genetic defects in Fas expression protect against tubular injury during ARF induced by ischemia-reperfusion or ureteral obstruction [12]. Changes in tubular Bcl-2-like proteins have also been noted. In murine obstructive-toxic ARF induced by an overdose of folic acid, tubular expression of Bax and Bcl-x_L is increased and Bcl-2 decreases [23]. These changes are transient (24-72h) and reminiscent of those observed during a well characterized model of epithelial apoptosis: involution of the mammary gland after weaning. Immunohistochemistry showed that tubular cells with upregulated Bcl-x_L expression coexist with cells that had lost the basal Bcl-x_L expression (Fig. 4). The competence for survival factors may explain that tubular cells with access to survival factors upregulate Bcl-x_L, and those deprived of them downregulate Bcl-x_L [23]. Bax was localized to apoptotic cells in the tubular wall and lumen in this model by immunohistochemistry [23]. Tubular Bcl-2 has been reported to be increased in the rat ischemia-reperfusion model of ARF [38]. However, in this model the Bcl-2 increment was restricted to the first few days after injury, while Bax was increased both on day 1 and days 7, resulting in a decreased Bcl-2/Bax ratio at the time and location of apoptotic clearing of hyperplastic epithelium [38]. Information regarding the expression of apoptosis regulatory proteins in human native kidney ARF is

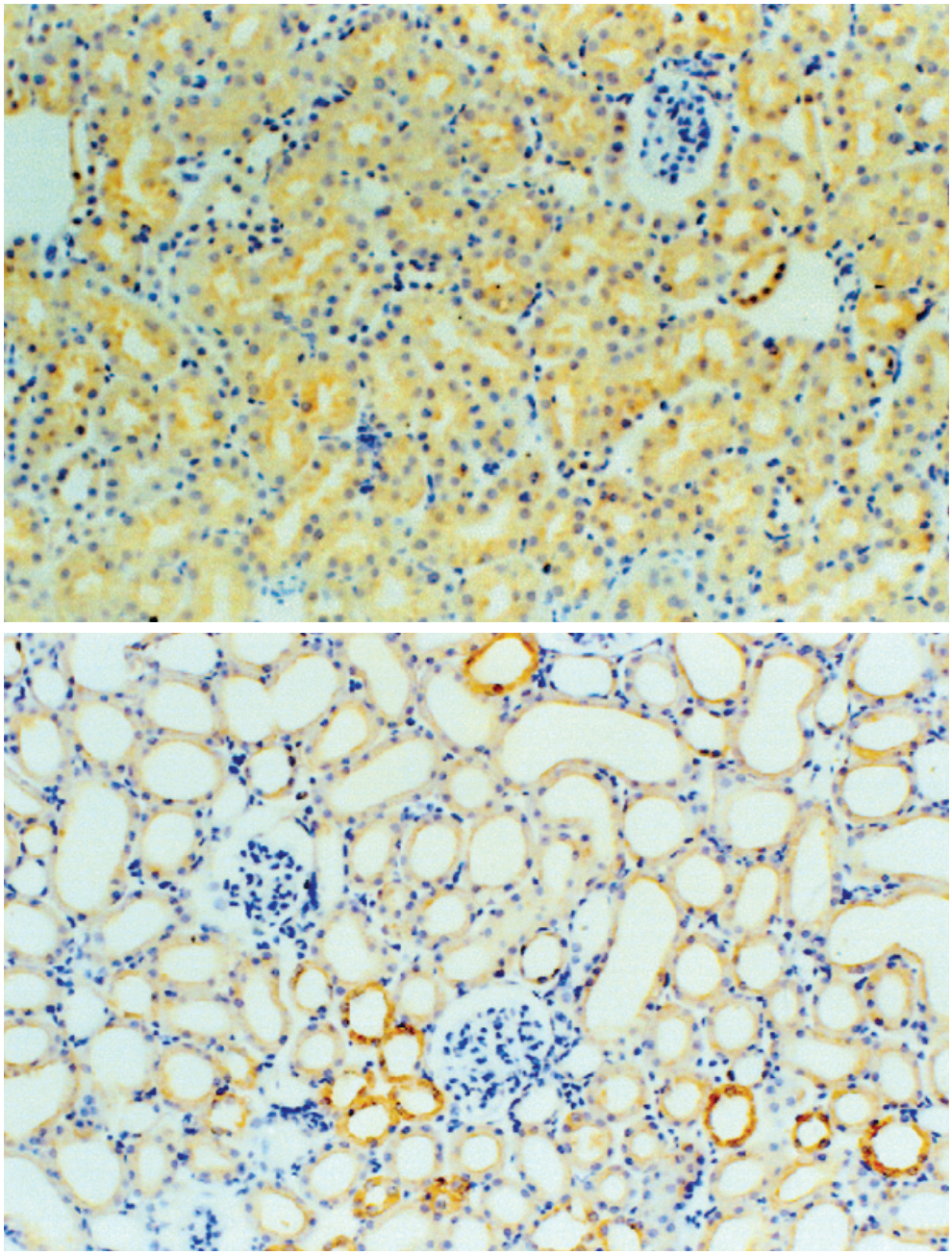

Fig. 4 Expression of apoptosis regulatory genes in experimental acute renal failure. Diffuse expression of Bcl-x_L in tubular epithelial cells in normal murine kidney (upper panel). During acute renal failure there is upregulation of Bcl-x_L expression in some tubules and downregulation in others (lower panel). Original magnification x400. See also [23].

needed. During acute transplant rejection an increased tubular apoptotic rate was associated with increased renal expression of lethal factors (perforin/granzyme B, FasL) and increased tubular expression of Fas and p53, and decreased Bcl-2/Bax ratio [39].

Less information is available regarding apoptosis regulatory factors in **chronic progressive tubulointerstitial injury**. Increased tubular Fas expression has been noted in mouse models of chronic renal atrophy [14]. Although glomerular cell apoptosis is not a feature in diabetic nephropathy, tubular cell apoptosis associated with decreased renal Bcl-2 and increased bax mRNA expression was observed [28]. This intracellular milieu may also predispose to ARF. Changes in apoptosis regulatory

proteins have also been observed in experimental nephrosclerosis and reduced renal mass [34,36].

Altered apoptosis appears to have a role in **PKD**. Apoptosis is required for cystogenesis in an *in vitro* system in canine MDCK cells and prevention of apoptosis by overexpression of Bcl-2 inhibits cystogenesis [40]. Consistent with this report, the lack of functional Bcl-2 results in excessive renal apoptosis and hypoplastic kidneys with a reduced number of nephrons and PKD [40]. The protein encoded by the PKD1 gene protects MDCK cells from apoptosis and prevents cystogenesis [41].

The prospects for therapeutic intervention

Current evidence suggests that apoptosis and its regulatory molecules contribute to a variety of renal diseases. Understanding the role and regulation of apoptosis in renal disease has the potential to provide the basis for the design of new therapeutic strategies as well as to improve our understanding of the mode of action of current therapies. Future research should focus on the definition of the cellular and molecular targets as well as the optimal time frame for therapeutic intervention in each renal pathology. Special consideration should be given to optimizing modes of local delivery of apoptosis modulatory therapies so as to target only specific cell populations during a limited period of time. Otherwise, we risk interfering with physiological apoptosis taking place during renal healing or in other organs and thus induce untoward effects. For instance, the systemic antagonism of Fas can result in autoimmunity in mice. A theoretical risk of promoting neoplasia can be predicted if there are no temporal limits to Bcl-2-like survival protein overexpression. It should be remembered that Bcl-2 was originally characterized as an oncogene. Conversely, attempts to increase the clearance of unwanted mesangial or tubular cells can be complicated by renal atrophy.

Among the cellular targets, we might be interested in prolonging parenchymal cell survival in chronic renal atrophy and ARF. Specific targeting of Bcl-2-like survival proteins may be of value. Studies in tubular epithelial cells have demonstrated the feasibility and effectivity of gene transfer strategies to promote the expression of Bcl-2 or Bcl-x_L and prevent tubular epithelium apoptosis *in vitro* [23]. Inhibition of caspases also protects from cell death mediated by death receptors *in vivo*. In laboratory animals the systemic administration of zVAD decreased acute tubular injury [37].

Conversely, leukocytes and fibroblasts may be targeted with proapoptotic maneuvers, besides the nonspecific lymphocyte apoptosis induced by corticosteroids and cyclosporine A. Other approaches may be to improve disposal of the apoptotic cells, which would prevent their lysis and limit inflammation and the release of autoantigens.

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