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ROLE AND REGULATION OF APOPTOTIC CELL DEATH IN THE KIDNEY. Y2K UPDATE

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1. ABSTRACT

Apoptosis is an active form of cell death that, in balance with mitosis, regulates cell number. Cell number abnormalities are a frequent feature of renal disease. We now review current concepts on the molecular regulation of apoptotic cell death, including the influence of survival and lethal factors from the extracellular microenvironment as well as the role of intracellular regulators of apoptosis, such as death receptors, proapoptotic and antiapoptotic bcl2-related proteins, the mitochondria and caspases. In addition the role of apoptosis in the genesis, persistence and progression and remodeling and resolution of renal injury is discussed. Information on the expression and function of apoptosis regulatory proteins in specific renal syndromes is summarized. Finally, future perspectives in research and clinical intervention are discussed.

2. CELL NUMBER REGULATION: MITOSIS AND CELL DEATH

Tissue cell number is carefully regulated through the balance between cell birth (mitosis) and cell death, with occasional participation of cell migration or cell trans-differentiation. Cell birth and cell death are intertwined and their rate frequently increases or decreases coordinately. Cells that die are frequently those that were recently born. This suggests that there are common mechanism that regulate cell birth and cell death, some of which have been identified. 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).

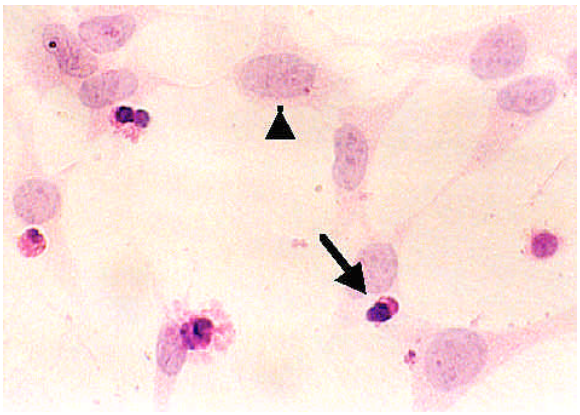

Figure 1. Pyknotic nuclei are small condensed nuclei from apoptotic cells. Note pyknotic nuclei and shrunken cytoplasm in murine tubular epithelial cells exposed to 300 µg/mL acetaminophen for 24h (arrow), when compared to a healthy cell (arrowhead). (H&E, original magnification x400).

3. CELL DEATH: APOPTOSIS AND NECROSIS

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 (1). 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 (2-9). 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. About 100000 cells are produced every second in a human, and a similar number die through apoptosis (3). 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 (Figure 1). DNAses fragment DNA in internucleosomal sized fragments, that yield a typical ladder pattern when separated by gel electrophoresis. Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) allows the in situ detection of fragmented DNA in individual cells in tissue sections. This technique requires great expertise, as false positive and false negative results are common, and simultaneous identification of the typical morphological features is recommended. 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. The term oncosis has been proposed for this form of cell death but is not widely employed. In this nomenclature, necrosis would denote the final stage of cell death that has proceeded either through oncosis or apoptosis (secondary necrosis)(1).

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 (3). 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 (10,11). 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 Bcl2 and BclxL protect against cell death with morphology of either apoptosis or necrosis (6).

4. MOLECULAR REGULATION OF APOPTOSIS

Corresponding to its importance in cell number homeostasis, apoptosis is a tightly regulated phenomenon. Both extracellular and intracellular molecules provide multiple regulatory and contrarregulatory pathways. We will review the current understanding of apoptosis regulation, making reference to renal cells when such data are available.

4.1 REGULATION OF CELL DEATH BY 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.

4.1.1. Survival factors

Most cells need survival signals from their surroundings to remain alive. However the survival factors

Table 1. Influence of the microenvironment on renal cell survival

Agent	Survival factors	Lethal factors
Cytokines	• IGF-1 (MC, TEC, RF) • IGF-II (MC) • EGF, proHB-EGF, HGF (TEC) • bFGF (RF) • PDGF (RF)	• TNF (MC, TEC, RF, RE) • FasL (MC, TEC, RF, RE) • IL-1α (TEC)
Extracellular matrix	• Integrin-mediated (TEC) • Clusterin (TEC) • Collagen IV, laminin (MC)	
Lipids	• Lysophosphatidic acid	• Thromboxane A2
Small molecules		• Oxygen radicals, H_2O_2 (MC, TEC) • Nitric oxide (MC,TEC, RE)
Drugs		• Cyclosporin A, cisplatin, acetaminophen, • aminoglucosides (TEC), HMGcoA inhibitors (MC)
Physical factors		• Pressure (MC)
Infectious agents		• Verotoxin, HIV (TEC)

MC: mesangial cells, TEC: tubular epithelial cells, RF: renal fibroblasts, RE: renal endothelium.

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 TGFß1 had no effect (12). 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 ß1-integrin-mediated mechanism (13). By contrast, pathological components of the glomerular ECM, such as collagen I and fibronectin offered no protection (13). Survival factors for tubular epithelium and renal fibroblasts are summarized in Table 1 (14-18).

Clusterin (SGP40) was one of the earliest proteins whose expression was associated with the occurrence of apoptosis, although for years its role in the process was unknown. It has now become evident that clusterin is a multifunctional protein that protects from apoptosis in vitro and in vivo (19,20). Exogenous clusterin prevents H_2O_2 cytotoxicity in tubular cells (20). In mesangial cells clusterin is downregulated by lethal stimuli such as serum deprivation and TNF (21).

4.1.2. Lethal factors

There are two kinds of lethal factors that induce apoptosis: those that activate specific cell death receptors and those that damage the cell in the absence of receptor activation.

4.1.2.1. Lethal cytokines

Lethal cytokines belonging to the TNF superfamily bind to and activate cell membrane death receptors (5) (Table 2). 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 (Table 2)(5). 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 (5,22).

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 (22,23). The main source of intrinsic FasL is the tubular epithelial cell (24). TNF and FasL can induce apoptosis of mesangial cells, tubular epithelial cells, renal endothelial cells and renal fibroblasts (25-29). 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 (28-30), as expected by their constitutive expression of the cytokine. However, they become sensitized to FasL lethality upon exposure to inflammatory cytokines (28,31). By contrast Fas activation induces death in non-stimulated mesangial cells in vitro and in vivo (27-29), and Fas-induced death is increased by inflammatory cytokines (27-28).

While Thy-1 is not considered a death receptor, anti-Thy-1 antibodies induce apoptosis of mesangial cells that appears to be the result of activation of this receptor (32).

4.1.2.2. Other extracellular lethal factors

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. Table 1 summarizes lethal stimuli for renal cells (10,11,33-39).

4.1.3. 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

Table 2. Lethal cytokines and death receptors

Cytokine	Receptor	Soluble or decoy receptors
TNF	TNFR1	sTNFR1
Apo-1L/FasL/ CD95L	Apo-1/Fas/CD95	sFas DcR3
Apo-2L/TRAIL	DR4/TRAILR1DR5/ TRAILR2/Apo-2/ Killer/TRICK2	DcR1/TRAILR3 DcR2/TRAILR4
Apo-3L/TWEAK	DR3/TRAMP/ WSL-1/AIR/LARD	?
?	DR6	?
LT-1	?	?

Table 3. Mammalian caspases

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

Table 4. Bcl2-family proteins and their role in apoptosis

Survival proteins	Proapoptotic proteins
• Bcl2*	❑ Bax subfamily
• BclxL*, **	❑ Bax
• Bclw	❑ Bak
• Boo	❑ Bok/Mtd
• A1	❑ BclxS**
• Mcl1	❑ Diva
	❑ BH3-domain-only subfamily
	❑ Bid
	❑ Bad
	❑ Bik/Nbk/Blk
	❑ Bim/Bod
	❑ Hrk/DP5

*Caspase-generated Bcl2 and BclxL fragments may promote cell death, ** BclxL and BclxS are isoforms derived from alternative splicing of the same gene.

cytokines and nephrotoxic drugs. The survival factors IGF-1 and IGF-II, but not bFGF, protected mesangial cells against DNA-damaging agent and protein synthesis inhibitor-induced apoptosis (12). However neither of them prevented Fas-induced apoptosis (12). By contrast, the survival factors present in serum or single cytokines such as IGF-1 protected murine tubular epithelial cells and renal fibroblasts from TNF- and Fas-induced apoptosis (18,40). Serum also protected tubular cells against apoptosis induced by nephrotoxins (41). Serum is frequently used as a source of survival factors in cell culture experiments. Other lethal stimuli, such as lovastatin, induce apoptosis only in actively proliferating mesangial cells and spare quiescent cells grown in serum-free conditions (39). This property could be used therapeutically to target proliferating mesangial cells in vivo.

4.2. INTRACELLULAR REGULATION OF APOPTOSIS

The intracellular regulation of apoptosis is one of the forefront fields in biomedicine research. A complete review is beyond the scope of this text, and we will briefly summarize current knowledge in the field. We must stress that 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 intracellular regulation of apoptosis is highly conserved in the philogenetic scale, and mammalian proteins function in invertebrates and viceversa. Seminal studies in the nematode C. elegans defined a basic role for CED-3, CED-4, CED-9 and EGL-1 proteins in apoptosis (Figure 2)(3). CED-3 and CED-4 are required for the execution of cell death. CED-4 associates with CED-3 and promotes the proteolytic activation of CED-3. CED-3 activation is inhibited by CED-9. EGL-1 binds to and inhibits CED-9 by disrupting the association between CED-4 and CED-9, thus promoting CED-3 activation (3).

CED-3 is homologous to the mammalian caspases. Caspases are intracellular cysteine proteases that cleave their substrates after an aspartate residue (8)(Table 3). While some caspases are largely responsible for the proteolytic processing of proinflammatory cytokines, others are directly involved in the execution of apoptosis. Caspase-1 was the first caspase described. Later its role in apoptosis was shown to be marginal: its main known function is processing of IL-1ß. CED-4 is homologous to Apaf-1 and FLASH, nucleotide-binding oligomerization domain containing proteins (42). They function as chaperones to increase the local concentration of procaspases and facilitate their autocatalytic activation.

CED-9 has significant homology to the mammalian Bcl-2 family of survival proteins (Table 4).This family also includes proapoptotic members (6). EGL-1 is a BH3 domain-containing protein most homologous to proapoptotic members of the Bcl2-family (Table 3)(3).

While the main or only known function of the above mentioned protein families is regulation of cell death, some of the signal transduction pathways that participate in cell death have important roles in other cellular processes and it is difficult to classify them as cell death factors.

4.2.1. Activators of apoptosis

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 Bcl2 family and mitochondrial injury (Figure 3).

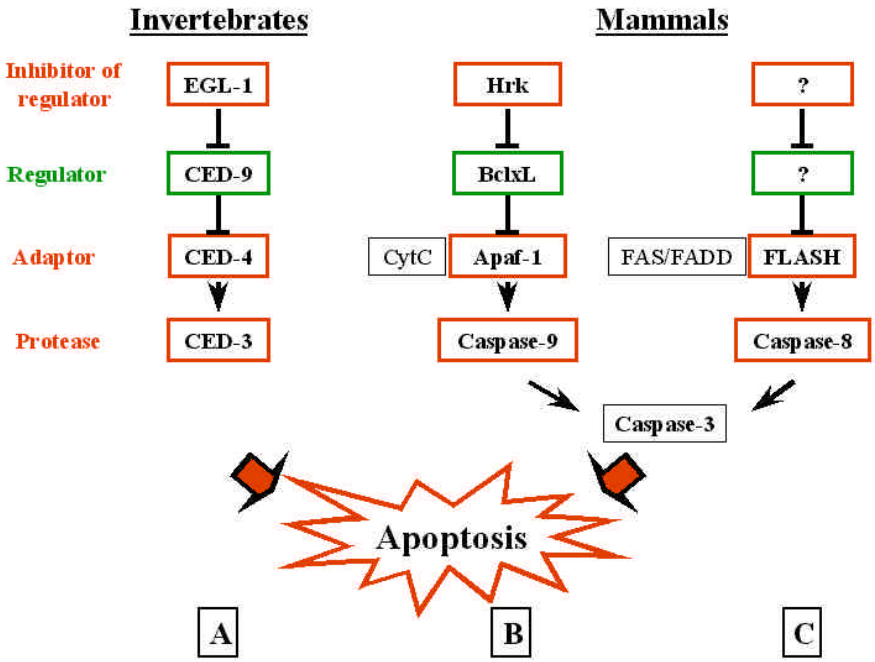

Figure 2. Intracellular regulation of apoptosis: A. Invertebrates. B. Mammals: Mitochondrial pathway for apoptosis. C. Mammals: Death-receptor initiated apoptosis.

4.2.1.1. Death receptors

Death receptors belong to the TNF receptor (TNFR) superfamily, which is characterized by homology in the extracellular domains. In addition, death receptors contain a cytoplasmic death domain (DD). 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 (5). A CED-4-like protein, FLASH, is also recruited to the activated Fas receptor and interacts with procaspase-8 via a DED domain (42). 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 in a variety of situations (43), 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 (5).

Type I and type II are differentiated according to the efficiency in forming a death signaling complex upon FasL binding to Fas (5). 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 but there are little data regarding their expression of soluble Fas and decoy receptors (22). 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 (11,22,28,31,33). Plasmas from patients with thrombotic microangiopathy induces apoptosis and Fas expression in renal microvascular endothelial cells (26).

CD27, a member of the TNFR family, can also induce apoptosis. Unlike TNFR1 and Fas, the cytoplasmic tail of CD27 lacks the DD. However, another protein that binds to the CD27 cytoplasmic tail, Siva, has a DD homology region and promotes apoptosis (44).

4.2.1.2. Proapoptotic Bcl2-like proteins

Some members of the Bcl2-family can trigger apoptosis (Table 4). For example, Bax overexpression induces caspase-independent cell death and Bax is required for certain modes of cell death, such as survival factor deprivation in neurons (6). Mechanisms for their pro-apoptotic activity include: 1) binding and inhibition of Bcl2 or BclxL, 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 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 (37,39,40). TNF and LPS increased Bak and induced apoptosis in glomerular endothelial cells (25).

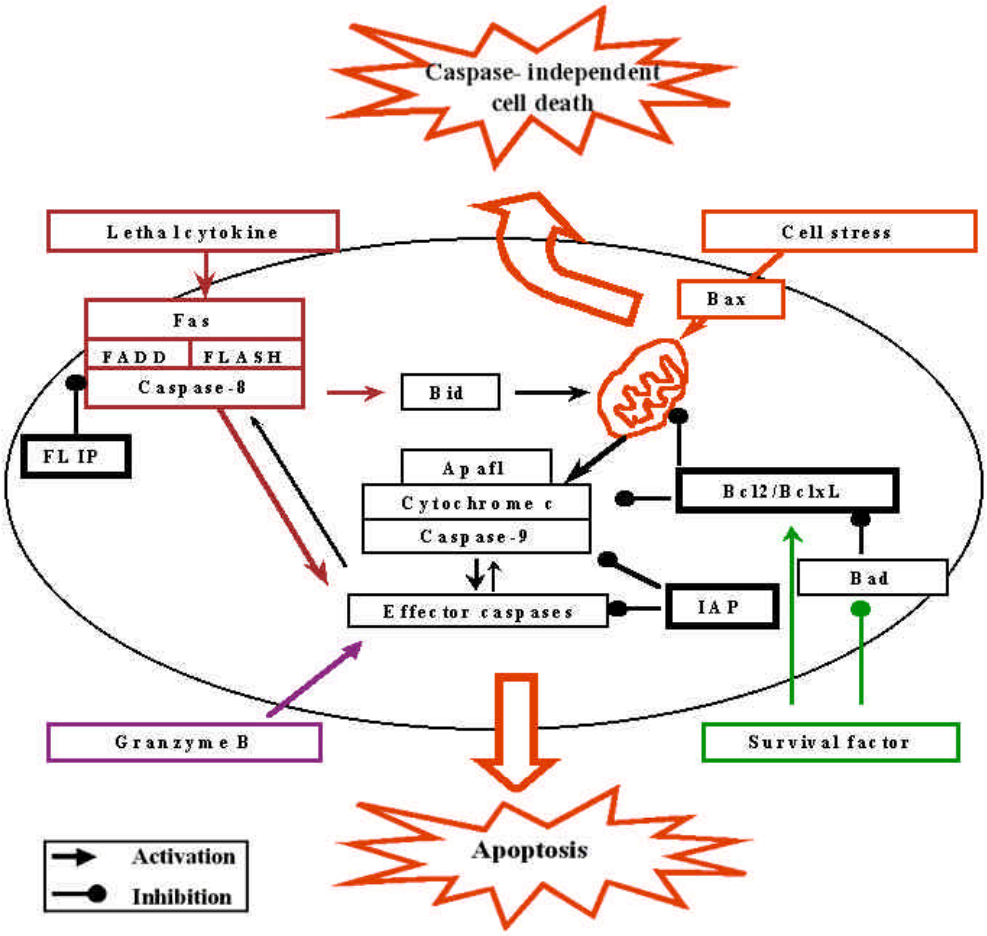

Figure 3. Intracellular pathways for cell death. Death receptors, cell stress or granzyme B can activate cell death pathways, and extracellular survival factor promote the activity of intracellular antiapoptotic factors. Mitochondrial injury can result in caspase-independent cell death without features of apoptosis.

A subfamily containing only the BH3 domain (BH3-only proteins) has recently been characterized (Table 4). Bad can bind to and inhibit BclxL and its ability to do so is downregulated by phosphorylation after survival receptor engagement by cytokines (3). Bid is the molecular connection between receptor-induced apoptosis and the mitochondria (4).

4.2.1.3.The mitochondria in apoptosis

Mitochondria are key participants in apoptosis that is not triggered by death receptors, and may also contribute to cell death after death receptor activation (9). 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 and AIF, from the mitochondrial intermembrane space to the cytosol, where they participate in the effector phase of apoptosis by directly activating caspases (9). 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 (9).

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)(4). Cyclosporin A inhibits ANT and protects against certain modes of apoptosis (4).

4.2.2. The executioner: caspases

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 3)(8). Alternatively, proteases, such as granzyme B, that are introduced into the cell by perforin-expressing cytotoxic lymphocytes activate caspases (4).

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 (4). 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 (8). 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 Bcl2 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 (8). Over 40 substrates for caspases have been identified, whose cleavage 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 BclxL and Bcl2, that may even yield proapoptotic fragments, dismantling of structural proteins and activation of DNAses (8).

Caspases may be classified according to their sequence specificity (8). Caspases with similar cleavage specificity share inhibitors. Among the synthetic peptide inhibitors of caspases, YVAD inhibits caspase-1-like caspases; DEVD, caspase-3-like caspases; IETD, caspase-8-like caspases; and zVAD inhibits all known caspases. There are also viral inhibitors of caspases whose antiapoptotic properties might be used therapeutically. They include the cowpox virus CrmA serpin and the baculovirus p35 protein. CrmA inhibits mainly caspase-1 and displays the second highest affinity for caspase-8. It is unlikely that crmA inhibits other caspases in vivo. In addition, crmA also inhibits granzyme B and other serine proteases. p35 inhibits all caspases tested so far. 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 (5,8,45).

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. zVAD inhibits the features of apoptosis induced by acetaminophen or hypoxia-reoxygenation in renal cells, but it does not prevent eventual cell death (37,46). 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 (8). There is yet not enough information on which caspases are important in mediating renal cell death. Studies employing inhibitors of caspases suggest that different caspases may be required for apoptosis induced by different stimuli. For example, DEVD-CHO prevents cyclosporin A, but not acetaminophen-induced apoptosis in murine tubular epithelial cells (34).

Other proteolytic enzymes also become activated during apoptosis, such as calpaine, a calcium-dependent cysteine protease, implicated in hypoxia-induced death of rat proximal tubules (47).

4.2.3. Protective proteins: survival Bcl2 family members

Survival Bcl2-like proteins (Table 4) protect from cell death in which the mitochondrial pathways for apoptosis are activated (6). 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 BclxS) and antiapoptotic (such as Bcl2 and BclxL) proteins (6). 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 Bcl2 family members is still been debated. Two theories have emerged. In one apoptosis is prevented by sequestering procaspases in the apoptosome. In this sense, BclxL binds to the complex formed by caspase-9, Apaf-1 and cytochrome c and prevents caspase activation (6). A second scenario involves closing the VDAC and preventing the release of mitochondrial apoptogenic factors such as cytochrome c and AIF into the cytoplasm (48). This effect may be related to the ability of these proteins to form transmembrane channels (6).

Modulation of Bcl-2 like proteins by extracellular survival and lethal factors has been described in renal cells. The survival factors in serum upregulated Bcl2 and BclxL expression and downregulated Bax in murine tubular epithelial cells, while lethal factors such as TNF and acetaminophen downregulated BclxL (40,41). TNF and LPS also downregulated BclxL in glomerular endothelial cells (25). High glucose concentrations, similar to those found in diabetes, upregulated bax mRNA and downregulated bcl2 and bcxL mRNAs in tubular epithelial

cells (49). Although neither survival cytokines nor ECM modified these proteins in mesangial cells (12,13), a NO donor and oxygen radicals upregulated Bax expression in mesangial cells (38). Bax upregulation in this system may be related to p53 activation (38).

Downregulation of BclxL expression may indeed have a role in tubular epithelial cell death, as preventing the fall in BclxL levels in transfected cells affords protection against apoptosis induced by nephrotoxins and lethal receptors (40,41). This later feature suggests that tubular epithelial cells are type II cells. Overexpression of Bcl2 also protected tubular epithelial cells from apoptosis induced by cisplatin or hypoxia-reoxygenation (35,37).

Heat shock proteins also protect from apoptosis. In mesangial cells heat shock protein 70 (Hsp70) overexpression protected from IL-1ß mediated sensitization to apoptotic stimuli (50).

4.2.4. Intracellular 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 (4-9). 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)(3). 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 (12,28). 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 (7). Interestingly, TNF induces both c-myc expression and apoptosis in serum-deprived tubular epithelial cells (40). p53 promotes apoptosis through pathways that can be both independent and dependent on the transcriptional downregulation of Bcl2 and upregulation of Bax (7). NFκB is activated upon engagement of some death receptors (5). One function of NFκB is to prevent cell death, as it is the case in mesangial cells exposed to TNF (51). AP-1 has both proapoptotic and antiapoptotic effects (52). Increased expression of transcription factors has been noted in the course of proliferative glomerular injury and tubular damage, where their contribution to the regulation of apoptotic cell death is difficult to evaluate.

4.3. 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 (7). 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. Indeed, p27Kip1 -/- mice develop more intense tubular proliferation and apoptosis following ureteral obstruction as well as more severe glomerulonephritis (53). Overexpression of Bcl2 or BclxL protects from apoptosis and also decreases cell proliferation (6).

5. 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 (2).

5.1. ROLE OF APOPTOSIS IN RENAL DISEASE

Both apoptosis of intrinsic renal cells and of infiltrating leukocytes may contribute to the pathogenesis of renal disease (Table 5).

5.1.1. Apoptosis as a mechanism of depletion of intrinsic renal cells

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.

5.1.1.1. Apoptosis as the initial insult that causes renal disease

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 (29,32,54). Agonistic anti-Fas antibodies cause complement-independent, acute, mesangial cell apoptosis, that is associated with transient proteinuria and hematuria (29). By contrast anti-Thy-1 antibodies cause complement-dependent mesangial cell apoptosis that is followed by self-limited proliferative glomerulonephritis (54). Tubular cell death in the early stages of ARF can proceed through apoptosis or necrosis (44,55-57). The relative contribution of the two mechanisms to the initial tubular cell loss is uncertain, and may depend on the severity of the insult.

4.1.1.2. Apoptosis as a contributor to tissue remodeling and resolution of renal injury

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

Table 5. Dual role of apoptosis in renal injury: pretty woman versus old witch

Cell type undergoing apoptosis	Beneficial consequences	Pathological consequences
Intrinsic renal cells:	Tissue remodeling:	Promote/initiate renal injury
Mesangial cells	Kidney development	Glomerulonephritis
Glomerular epithelial cells	Resolution of hypercellularity:	ARF
Tubular epithelial cells	Proliferative glomerulonephritis	Progression of renal injury
Vascular cells	Recovery from ARF	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 against apoptosis-generated autoantigens*

* These mechanisms have been invoked when apoptotic cell phagocytosis fails

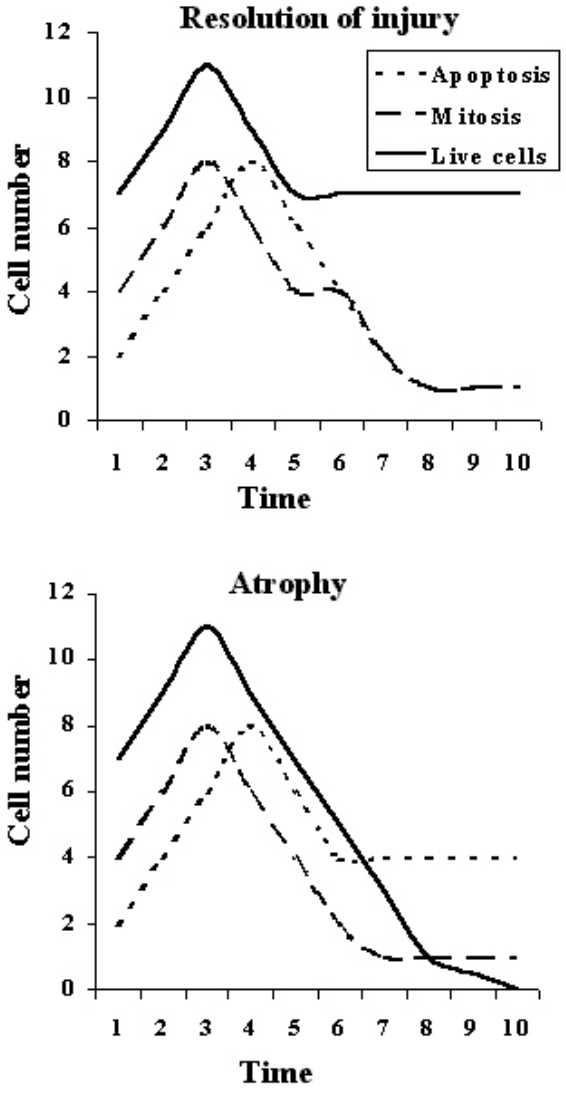

Figure 4. Cell number homeostasis results from the balance between mitosis sand cell death. While apoptosis of excess cells may result in restoration of normal cell number, if apoptosis persistently exceeds mitosis this can result in tissue atrophy.

can result in renal dysplasia or agenesis (14).

Evidence from experimental models of renal injury suggests that apoptosis coexists with renal cell proliferation. These cells may be more sensitive to absolute or relative deficits in survival factors. 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.

Cell turnover in the healthy glomerulus is low. Apoptotic cells represent about 0.01% of rat glomerular cells and 0.03/10 glomerular cross-sections in humans (58). Resolution of the self-limited mesangial proliferation characteristic of experimental anti-Thy-1 nephritis depends on a high rate of apoptosis of excessive mesangial cells, that exceeds the rate of mitosis and peaks at 0.25% of glomerular cells (58). However the rate of apoptosis returns to basal levels before leading to glomerular hypocellularity (Figure 4). By contrast the rate of apoptosis was not increased in non-proliferative glomerulonephritis such as experimental membranous nephropathy (58).

A second peak of apoptosis occurs days to weeks (it peaks at day 8 in rat ischemic ARF) after the original tubular insult that led to ARF, when the necrotic tubules have been completely reconstituted by a hyperplastic epithelium (44,55). In this setting apoptosis may restore cell number to pre-injury levels, but it may also contribute to delay recovery from ARF. This may be especially true in the clinical setting. Contrary to the experimental situation, in which a single insult causes enough damage for the model to be reproducible, the clinical setting is often characterized by repeated low grade offenses. This low grade renal damage leads to tubular epithelial cell apoptosis, even in the absence of any clinical deterioration of renal function (59) and may hamper recovery in previously damaged tubuli. Indeed the definitive interpretation of the clinical significance of this late apoptotic response awaits the results of therapeutic attempts to avoid the loss of tubular cells at this stage.

Insufficient fibroblast apoptosis can promote accumulation of interstitial fibroblasts during renal scarring, as fibroblasts involved in wound repair are eliminated by apoptosis (60).

5.1.1.3. Apoptosis in the persistence and progression of renal injury

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 glomerular sclerosis or tubular atrophy (Figure 4). 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 membrane antibody-induced nephropathy (61,62).

Consistent with the experimental data, an increased rate of apoptosis was observed in human proliferative nephritis such as IgA nephropathy and lupus nephritis, especially in sclerosing lesions (63). 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 (58,61,62).

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 (31,63-66).

5.1.2. Apoptosis in the regulation of inflammation in kidney diseases

Many renal diseases are characterized by a mononuclear cell infiltrate composed mainly of monocytes/macrophages and T cells. Inflammatory cells provide factors that cause parenchymal cell apoptosis. Monocytes/macrophages release TNF, FasL, oxygen radical species and NO. In contrast, the combination of perforin/granzyme B and FasL accounts for most of the cytotoxicity of T lymphocytes (58). Indeed, prevention of mononuclear cell recruitment in MCP-1 -/- mice decreased tubular cell apoptosis associated to glomerular inflammation (67).

Clearance of inflammatory cells by apoptosis contributes to resolution of renal inflammation (58) and failure of this clearance may contribute to the persistence of the inflammatory process. Apoptosis of neutrophils is prominent in nephrotoxic nephritis in the rat (58) and may be responsible for the high apoptosis rate observed in acute postinfectious glomerulonephritis in humans (68). Apoptotic leukocytes are engulfed by local cells, like mesangial cells (58).

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 (1). 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 (58). In fact, inhibition of apoptosis resulted in decreased renal inflammation following ischemia-reperfusion (69).

5.1.3. Apoptosis and the immune response

Apoptosis of lymphocytes plays a fundamental role in the control of the immune response in the thymus and the periphery (70). While the details of this involvement are beyond the scope of this review, it should be noted that altered expression of apoptosis-related proteins such as Bcl-2, Fas and FasL results in autoimmunity and renal damage (70). Apoptosis by itself may generate autoimmunity, as autoreactivity has been recognized against antigens present in apoptotic cells (70). If apoptotic cells are not adequately cleared, their contents might be released and further stimulate this autoimmune response.

5.2. APOPTOSIS REGULATORY PROTEINS IN RENAL DISEASE

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 human biopsies, 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).

5.2.1. Glomerular injury

In concordance to the observation of increased rates of apoptosis in proliferative glomerulonephritis, 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 (22,23). Increased expression of Fas, Bcl-2 and Bax has been observed within the glomerulus in human proliferative glomerulonephritis (71,72). However, Bcl2 positivity was limited to less than 2 cells/glomerulus and it could represent expression by infiltrating leukocytes (71). Other authors did not find significant Bcl2 immunoreactivity

within the glomerular tuft in a variety of proliferative and non-proliferative glomerulonephritis, the expression being limited to epithelial cells in early crescents (73).

5.2.2. Acute renal failure

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 (EGF, IGF-1) and increased cell membrane survival factor receptors, indicating competition for survival factors (16). Increased TNF and FasL have also been observed in ARF and are matched by high renal Fas expression in experimental models of ARF (22,23,28). Furthermore, genetic defects in Fas expression protect against tubular injury during ARF induced by ischemia-reperfusion or ureteral obstruction (22,23). Siva mRNA and CD27 receptor were expressed both early after post-ischemia as well as in epithelial cells of regenerating tubules, in association with the two peaks of apoptosis (44).

Changes in tubular Bcl2-like proteins have also been noted. In murine obstructive-toxic ARF induced by an overdose of folic acid, tubular expression of Bax and BclxL is increased and Bcl2 decreases (40). 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 BclxL expression coexist with cells that had lost the basal BclxL expression. These findings are consistent with data from central nervous system ischemia, where BcxL was increased in surviving neurons and decreased in damaged neurons (74). The competence for survival factors may explain that tubular cells with access to survival factors upregulate BclxL, and those deprived of them downregulate BclxL (40). Bax was localized to apoptotic cells in the tubular wall and lumen in this model by immunohistochemistry (40)..

Tubular Bcl2 has been reported to be increased in the rat ischemia-reperfusion model of ARF (75). However, in this model the Bcl2 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 Bcl2/Bax ratio at the time and location of apoptotic clearing of hyperplastic epithelium (75).

Information regarding the expression of apoptosis regulatory proteins in human native kidney ARF is 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 Bcl2/Bax ratio (76-78).

5.2.3. Chronic tubular atrophy and renal fibrosis

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 (31). Although glomerular cell apoptosis is not a feature in diabetic nephropathy, tubular cell apoptosis associated with decreased renal bcl2 and increased bax mRNA expression was observed (49). This intracellular milieu may also predispose to ARF. Interestingly, apoptosis in blastocysts exposed to high glucose levels depends on increased Bax (79).

5.2.4. Polycystic kidney disease

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 Bcl2 inhibits cystogenesis (80). 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 (reviewed in 81). PKD also develops in other mouse models with genetic defects in apoptosis related genes. Dysregulation of c-myc in the kidneys of SBM mice results in PKD and increased proliferation and apoptosis, in the absence of changes in Bcl2 or Bax expression. AP-2ß -/- mice die postnatally because of PKD that is associated with massive tubular apoptosis.

The proteins encoded by the PKD1 and PKD2 genes may participate in interactions with extracellular ligands (81). I am not aware that the intriguing possibility that they influence cell survival or death has been formally addressed yet. Human autosomal dominant PKD (ADPKD) is characterized by increased c-myc expression in cysts, as well as increased rates of proliferation and apoptosis, despite increased Bcl2. Apoptosis has also been implicated in the loss of normal renal parenchyma in ADPKD.

6. FUTURE PERSPECTIVES IN RESEARCH AND CLINICAL INTERVENTION

Although incomplete, the available in vitro and in vivo data suggest 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. Newer therapies may include drugs that interfere with apoptosis, cytokines and their antagonists, antisense strategies, and local delivery of apoptosis regulatory genes. 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 (22). A theoretical risk of promoting neoplasia can be predicted if there are no temporal limits to Bcl2-like survival protein overexpression. It should be remembered that Bcl2 was originally characterized as an oncogene (6). 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. Survival cytokines and lethal cytokine antagonists have already been used in experimental renal disease. EGF and IGF-1 improve the evolution of experimental ARF and decreases tubular cell apoptosis (16,56). A more selective delivery of the survival factor could be envisioned. Gene transfer-induced overexpression of membrane-bound form of HB-EGF in tubular epithelial cells has a local antiapoptotic action (15). Antagonism of TNF improves the evolution of experimental models of ARF and glomerulonephritis (22,23). Although TNF induces apoptosis, the contribution of direct inhibition of apoptosis to the therapeutic effect of its antagonists is difficult to discern. Specific targeting of Bcl-2 like survival proteins may also be of value in the therapy of renal diseases. Studies in tubular epithelial cells have demonstrated the feasibility and effectivity of gene transfer strategies to promote the expression of Bcl2 or BclxL and prevent tubular epithelium apoptosis in vitro (35,37,40,41). Even though in vivo studies in the kidney are lacking, the local overexpression of Bcl2 in transgenic mice decreased cerebral infarct size by 50% (82). Local delivery of dominant-negative FADD constructs protects targeted cells from death receptor-mediated apoptosis (5). Inhibition of caspases also protects from cell death mediated by death receptors in vivo. In laboratory animals the systemic administration of zVAD prevented Fas-induced liver injury (83) and decreased the size of myocardial infarction by 20% when assessed at an early time point (84). By contrast, DNAse inhibition did not prevent cell death (85).

Conversely, leukocytes and fibroblasts may be targeted with proapoptotic maneuvers. In this sense, two of the most widely used immunosuppressive drugs, corticosteroids and cyclosporine A, promote lymphocyte apoptosis (58). Other approaches may be to improve disposal of the apoptotic cells, which would prevent their lysis and limit inflammation and the release of autoantigens (58). Corticosteroids foster the non-phlogistic disposal of apoptotic cells (86). The identification of cell-specific promoters will facilitate targeting of lethal genes, such as Fas or FADD, to excess fibroblasts (87).

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