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APOPTOSIS IN RENAL DISEASES

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1. ABSTRACT

Abnormalities of cell number are a frequent feature of renal disorders. Cell death is a key factor in the regulation of cell number. Apoptosis is an active form of cell death that is modulated by extracellular lethal and survival signals. Regulation of apoptosis also involves a complex system of sensors of the extracellular signals, triggers of the apoptosis program, effectors of apoptosis as well as intracellular survival factors. This paper first reviews current knowledge on the regulation of apoptosis with particular emphasis on renal cell death. Subsequently, it deals with the role of apoptosis in triggering renal disease and its participation in the progression and resolution of renal disorders. This section includes information on the occurrence of apoptosis and expression of apoptosis-related genes in glomerular injury, acute renal failure, chronic renal atrophy, renal fibrosis, polycystic renal disease and kidney development. The final section presents an overview of possible approaches to the therapeutic manipulation of apoptosis in the kidney.

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2. RENAL DISEASE AND CELL NUMBER

Homeostasis of cell number is the outcome of the coordination of cell birth (mitosis) and cell death. Physiological cell death usually takes place by apoptosis. Occasionally other processes, such as cell migration (chemotaxis) and differentiation, are also involved in the regulation of cell number.

Abnormalities of cell number are a frequent feature of renal disorders. Parenchymal cell depletion leading to renal atrophy is characteristic of any chronic, progressive, renal disease. Depletion of tubular cells is the hallmark of acute tubular necrosis. Other nephropathies are defined by an abnormal accumulation of renal cells. Examples may be mesangial hypercellularity in proliferative glomerulonephritis, and the increased number of fibroblasts observed in renal fibrosis. The possible role of altered mitotic rates in renal disease has been extensively studied. However, derangements in the regulation of cell death may also lead to diseases characterized by insufficient or excessive number of cells (1) and may contribute to renal damage (2-4). In the past 2 years, a flurry of papers have unraveled the intracellular regulation of apoptosis. We will first summarize the current knowledge on the regulation of cell death, with special emphasis on renal cells. Subsequently we will review evidence regarding changes in cell death regulation in renal disease.

3. APOPTOSIS AND PROGRAMMED CELL DEATH

The functional concept of programmed cell death implies an active participation of the cell in its own death (cell suicide) through the activation of a genetic program (5-7). In general, programmed cell death has the morphologic characteristics of apoptosis, although there are exceptions (7). In fact, there is functional, morphologic, and genetic evidence of heterogeneity in this process (6-9), and there are unanswered questions about the physiologic relevance of this diversity. In any case, the pragmatist may define programmed cell death (and because of the extensive use of the term, also apoptosis) as a process that can be modulated through interference with cell death related genes, independently of the morphology or pattern of DNA degradation. Thus, the main characteristic of apoptosis/programmed cell death would be its susceptibility to therapeutic intervention.

Apoptosis, however, is usually defined by a characteristic morphology and functional changes (10-12). Apoptotic cells display decreased cell and nuclear size with chromatin condensation, detachment from adjacent cells, cell membrane blebbing, and fragmentation with the formation of membrane-bound bodies (10).

Functionally, apoptotic cells express new cell membrane structures that determine a high rate of recognition and phagocytosis by adjacent cells. The integrity of the cell membrane is preserved for some time and cells are cleared before there is any significant leakage of pro-inflammatory molecules. The half-life of the apoptotic cell is a few hours (13, 14). As a consequence, a low percentage of apoptotic cells visible in a tissue section may be associated with a significant loss of cell mass (13). Apoptosis usually affects individual cells and its tissue distribution is patchy and asynchronous. Detection of apoptosis is further impaired in renal injury by the fact that detached apoptotic cells may be flushed away by urine (15).

Apoptosis requires the expression or suppression of certain genes (5). Endonucleases, tissue transglutaminases and proteases are activated (5,11,12). Some kinds of apoptotic cell death can occur in the absence of a nucleus if the required genetic machinery is constitutively expressed (16). In this regard, the inhibition of mRNA or protein synthesis may cause or prevent apoptosis (12), depending on the balance between lethal and protective factors in a given cell.

By contrast, necrosis is a passive mode of cell death that frequently involves fields of contiguous cells, and a prominent inflammatory response. Both apoptosis and necrosis can occur at the same time in the same tissue (17). The occurrence of either one may depend on the intensity of the precipitating events (18).

3.1 Extracellular factors in the regulation of apoptosis

Apoptosis may be the consequence of withdrawal of survival factors or exposure to lethal factors (1,19). The survival factor requirement may vary with cell type, functional status of the cell, or presence of lethal stimuli. Survival and death factors for extrarenal cells include cytokines, extracellular matrix, lipids, small molecules and microbial products. In addition, physical factors (such as heat, irradiation) and certain drugs may induce apoptosis.

3.1.1 Survival factors

Serum deprivation results in apoptosis of mesangial and tubular epithelial cells (20-22). Little is known about the specific factors that account for survival of renal cells. EGF prevents apoptosis in proximal tubular cells (14), and IGF-1 and bFGF in mesangial cells (23,24). By contrast, a survival activity could not be demonstrated for PDGF-BB and EGF in mesangial cells (24).

Extracellular matrix is a survival factor for epithelial (25) and mesangial cells (26), especially if soluble survival factors are not available. This effect is mediated by integrin receptors (26). The nature of the extracellular matrix is important. While basement membrane supports the survival of mesangial cells, type

I collagen (found in sclerotic but not in normal glomeruli) does not (26).

3.1.2 Lethal factors

Several cytokines and inflammatory mediators induce apoptosis. The cytotoxic effect of TNF-alpha on glomerular epithelial and mesangial cells in culture (27) has recently been characterized as apoptosis (20,28). IL-1-alpha is also lethal for mesangial cells (28). However, neither TNF-alpha nor IL-1-alpha induce glomerular endothelial cell apoptosis (28). Fas activation, oxygen radicals and anti-Thy1 antibodies also induce apoptosis of mesangial cells (29-31). TNF-alpha, Fas activation, inhibition of protein kinase C, nephrotoxins and ceramide induce apoptosis of tubular cells (18,21,32 and unpublished observation). Ceramide is a mediator of TNF-alpha and Fas-induced apoptosis (33). In tubular cells DNA degradation has been dissociated form the morphologic features of apoptosis (34)

3.1.3 Interaction of survival and lethal factors

Cell fate depends on the interaction of survival factors and apoptosis-inducing factors. In oligodendrocytes, ciliary neurotrophic factor (CNTF) prevents both growth factor deprivation-induced apoptosis and TNF-alpha-induced cell death (35). Several interleukins can rescue lymphocytes from glucocorticoid-induced cell death (36). Survival factor deprivation of renal tubular cells increases the susceptibility to apoptosis induced by TNF-alpha (21). While the basis for this interaction is unclear, it may depend on changes in the expression of apoptosis regulatory genes (21,37).

3.2 Intracellular regulators of apoptosis

The study of mutations of the genes involved in apoptosis in the development of *Caenorhabditis elegans* has identified two lethal genes, *ced-3* and *ced-4*, which are required for cell death, and a survival gene, *ced-9*, which prevents death (38). A third type of gene, *reaper*, encodes a protein that activates the death program in *Drosophila*, but appears not to be directly involved itself (39). In *C. elegans*, additional genes regulate later steps of the apoptotic process, such as cell engulfment and degradation (38).

In mammalians, genes that may be classified as sensors/triggers ("*reaper*-like") (40), effectors ("*ced-3/ced-4*-like")(41), and survival factors ("*ced-9*-like")(42,43) have been identified.

3.2.1 Sensors and triggers

Extracellular factors regulate cell survival and death through activation of sensors. The most intensively studied have been cytokine receptors. This sensors, in turn, could activate intracellular signals that trigger apoptosis.

3.2.1.1. Receptors that mediate cell survival and associated proteins

Integrins and cytokine receptors transduce poorly understood survival signals. The activity of the receptor depends not only on the availability of ligand, but also on intracellular regulators. EGF was one of the first cytokines shown to promote cell survival (14). The phosporylated EGF receptor binds to growth factor receptor-bound protein 2 (Grb2), which acts as a link to another intracellular signaling molecule, a guanine releasing factor of the son of sevenless (Sos) class. A Grb2 isoform, named **Grb3-3**, cannot bind to the EGF receptor, but does bind to the Sos-related factor (44). In this way, Grb3-3 acts as a dominant negative protein over Grb2. A direct functional consequence is that, when Grb3-3 is abundant, the survival promoting activity of EGF is blocked and apoptotic cell death is triggered (44). Both Grb2 and Grb3-3 are expressed in human kidney, although their possible role in renal physiology remains unexplored (44).

3.2.1.2. Receptors that mediate apoptosis and associated proteins

Several members of the TNF receptor superfamily regulate cell survival. This family is defined by similarities in their extracellular domains, and includes both TNF receptors, Fas, the NGF receptor and others (45). Both Fas (CD95) and 55 M*r* TNF receptor (TNFR1)-induced apoptosis requires the integrity of a relatively homologous intracellular domain (46,47). This so-called death domain defines a different family that includes receptors and cytoplasmic proteins that are involved in cell death (40). The 75 M*r* TNF receptor lacks the death domain but may also have a limited role in triggering cytotoxicity (48).

Agonistic anti-Fas antibodies, and the endogenous ligand for Fas induce apoptosis (49-53). Fas ligand is expressed mainly by T cells (53), but its transcript is also present in macrophages and cultured renal cells (29). Fas plays a role in T cell-mediated cytotoxicity and activation-induced T cell death (54-57). When the microenvironment is appropriate, Fas may activate lymphocytes (58). Thus, it is not surprising that genetic defects in Fas ligand (*gld* mice) and Fas (*lpr/lpr* and lpr^{cg} mice) result in autoimmunity and a lupus-like syndrome (59-61). Fas defects also cause autoimmunity in humans (62,63). Soluble Fas molecules may be secreted and bind to and antagonize the Fas ligand (64).

Human and murine mesangial and tubular cells express Fas (29,65). Bacterial endotoxin and cytokines thought to play a pathogenic role in kidney damage, such as TNF-alpha, IL-1-beta and IFN-gamma, increase *fas* mRNA and Fas receptor expression (29,65,65b). Fas receptor expression peaks at 48-72h after stimulation, even though increased mRNA is noted as early as 1 hour (65). This time course is similar to that previously described in thymocytes (66). Agonistic anti-Fas antibodies induce apoptosis in murine and

human renal cells (29 and unpublished). Death induced by Fas activation is increased in renal cells activated by cytokines or treated with the inhibitor of mRNA synthesis actinomycin-D (29). While the reason for the latter observation is not clear, several proteins protect against Fas-induced death, such as Fas-associated protein (FAP-1), a protein tyrosine phosphatase that associates to Fas (67).

The fact that Fas underexpression leads to autoimmunity and immune-mediated glomerulonephritis (60,61) does not necessarily exclude a role for Fas in renal damage. The genetic defect of *fas* in MRL-*lpr/lpr* lupus mice consists in the insertion of an endotransposon in the *fas* gene, leading to abnormal splicing and low levels of expression of normal *fas* transcript (61). These mice do however express the Fas receptor (68) and *fas* mRNA is detectable in the kidney (unpublished observation). Thus, the *lpr/lpr* mouse cannot be considered a knock-out for Fas. By contrast lpr^{cg} mice carry an inactivating point mutation in the death domain of the Fas molecule (60). A preliminary report suggests that mice carrying the lpr^{cg} gene in the MRL background display immune alterations equal to those of MRL *lpr/lpr* mice. However, even though glomerular C3 deposition is also similar, glomerular damage is milder in MRL-lpr^{cg} mice (69). One possible interpretation is that complete absence of functional Fas partially protects against glomerular injury in mice with autoimmunity.

Overexpression of Fas and of TNFR1 induces apoptosis in the absence of ligand, probably because of self-oligomerization through the death domain (70). Several cytoplasmic proteins share with these receptors the death domain and the ability to induce apoptosis through protease activation. They include the Drosophila protein reaper, mammalian receptor interacting protein (RIP), TNF receptor associated death domain (TRADD), and Fas associated death domain (FADD/MORT-1) (71-74). Beyond the similarity in the death domain, these proteins are unrelated. The death domain allows them to oligomerize and bind to the receptors. RIP and FADD bind to active forms of Fas, but not to mutated, inactive lpr^{cg} Fas (71,73,74). TRADD and, with less affinity, RIP, bind to TNFR1 (71,72). FADD appears to play a role in the transduction of the Fas death signal. It should be noted that the death effector domain in FADD is distinct from the so called death domain (74). RIP, TRADD and FADD are expressed in multiple organs, including the kidney (71-74).

3.2.1.3. Transcription factors

Several transcription factors have been implicated in apoptosis.

c-myc appears to activate a common genetic program that may determine both cell division and cell death. The presence or absence of additional signals (such as external survival factors or Bcl-2) may determine cell fate: the uncontrolled expression of c-myc increases cellular proliferation in the presence of growth factors, but in cells deprived of them, it induces apoptosis (75-77). c-myc activates the transcription of *p53* (78), and death induced by c-myc requires p53 (79,80). In serum-deprived mesangial and tubular cells *c-myc* mRNA is induced by stimuli that promote apoptosis such as TNF-alpha (37).

p53 is the most frequently mutated or deleted gene in solid neoplasia (81) and it also plays a role in benign processes: its expression is increased in relation to neuronal cell death during experimental cerebral ischemia (82). In thymocytes p53 is required for apoptosis induced by radiation and DNA damaging drugs, but not for dexamethasone-induced apoptosis (9,83). It also participates in survival factor deprivation induced apoptosis (84,85). The mechanism of cell death by p53 is unclear. p53 decreases the transcription of the antiapoptotic gene *bcl-2*, and increases that of its antagonist, *bax* (87,88). However, p53-induced apoptosis may be independent of transcriptional activation of p53-target genes (89). External survival factors or enforced Bcl2 expression protect cells from death induced by p53 (86,90).

Fos and **Jun** homo or heterodimerize to form the AP-1 transcription factor. The expression of c-*fos* and c-*jun* precedes apoptosis and is rapidly and transiently induced upon growth factor deprivation in IL-2 and IL-6-dependent cell lines (91). Antisense inhibition of either of them or the intracellular injection of antibodies protects against death (91,92).

Nur77 is required for TCR-induced apoptosis in T-cell hybridomas (93,94). The ability of cyclosporine A to interfere with this form of apoptosis may be related to its ability to block Nur77 binding to DNA (95). Similar to the other transcription factors already mentioned, Nur77 also plays a role in the cell cycle regulation. *nur 77* mRNA is expressed only after appropriate stimulation in cultured murine tubular and mesangial cells (unpublished observation) but it is so far unclear whether it regulates mitosis or death in these cells.

3.2.2 Effectors

Proteases are key mediators of the effector pathway of apoptosis induced by either survival factor deprivation or receptor activation (41). Several proteins, such as poly(ADP)ribose polymerase (PARP) are cleaved during apoptosis. Protease inhibitors, including the cowpox virus Crm A protein protect cells from death. Several Ced-3-related Cys proteases whose overexpression results in apoptosis have been identified in mammals (41).

The IL-1ß-converting enzyme (ICE) was the first to be identified as the protease involved in

apoptosis (96,97). ICE-induced cell death is prevented by CrmA and Bcl-2 (96). However, ICE-deficient mice do not display developmental abnormalities related to a defect in apoptosis, although Fas-induced apoptosis is defective (98). This suggests that either there is a redundancy in apoptosis effector pathways or that ICE is not the ultimate effector. In this regard, ICE cleaves PARP with low efficiency (99). ICE expression in cultured renal cells is low (unpublished observation).

Yama/CPP32ß/apopain is distal in the apoptotic pathway to ICE (100,101). Yama is a zymogen that, when activated, displays a CrmA-sensitive, Asp-specific Cys protease activity and cleaves PARP (100,101). However, in the absence of cytosol, Yama does not provoke apoptotic changes to nuclei, suggesting the involvement of other components of the apoptotic machinery (101). *Yama* mRNA is readily detected in both cultured renal cells and whole kidney (unpublished observation).

Related enzymes include Ich-1/Nedd2 (102), Ich-2/Tx/ICE-related protein II (103,104), Mch-2 and ICE-related protein III (105). Some are present as zymogens that are activated by proteolytic cleavage. The relationships between them are slowly being unraveled. Thus, Tx/Ich-2 can process pro-TX and pro-ICE (103), ICE is capable of processing both pro-ICE and pro-Yama to their active forms, but Yama cannot exert this function (100,106). This picture is further complicated by the fact that isoforms of these proteins have antagonistic effects on cell death. For example, Ich-1L promotes cell death, while Ich-1S inhibits this effect, presumably because it competes for the same targets (102). The main transcript expressed in kidney and cultured renal cells is *Ich-1L* (102 and unpublished).

Studies with protease inhibitors have implicated calpain I in apoptosis (107). Calpain cleaves and activates IL-1-alpha. The 17kD C-terminal fragment is referred to as mature IL-1-alpha, and is released to the medium. The 16 kD N-terminal fragment has recently been shown to be targeted to the nucleus and to induce apoptosis (108).

3.2.3 Survival proteins and regulatory factors

Among the proteins related to Ced-9 (Bcl-2, Bcl-xL, Bax, Bad, Bak), Bcl-2 and Bcl-xL protect cells from apoptosis, although their precise mechanism of action is unknown. Another set of proteins interact with the survival proteins, and may enhance or inhibit their survival promoting activity.

3.2.3.1. Survival proteins

Bcl-2 is a membrane-bound protein present in mitochondria and other intracellular membranes (109,110). It has been suggested that Bcl-2 interferes with lipid peroxydation or the production of reactive oxygen species (111,112), but this has not been conclusively proven (113,114). Bcl-2 affords cells partial or complete protection from death induced by survival factor deprivation, Fas, TNF-alpha, c-myc, p53, ICE, Ich-1, Yama, glucocorticoids, oxidants, phospholipase A_2, toxins, hypoxia and physical factors such as heat shock and irradiation (100,102,113-115 and reviewed in 116). The survival promoting potential of Bcl-2 has been demonstrated in renal cells (117). Bcl-2 does not protect against all forms of cell death. In some cases this has been explained by the need that this protein has to work in unison with other associated proteins (115).

Renal cells, including mesangial cells, tubular epithelium, fibroblasts and metanephric stem cells, express *bcl-2* mRNA and protein (21,118). Murine renal cells express *bcl-2* mRNA transcripts of several sizes (7.5, 4.1 and 2.4 kb); the 7.5 kb transcript being the most abundant. Gene expression of *bcl-2* in renal cells appears to be controlled by environmental factors that regulate cell survival (21). *In vivo*, Bcl-2 protein is more abundant in embryonic than in adult kidney (119,120).

Derangements in Bcl-2 expression are associated with renal disease. For example, over-expression of Bcl-2 in B-lymphocytes of transgenic mice is associated with autoimmunity and the development of a proliferative glomerulonephritis (121). Mice carrying a targeted mutation in *bcl-2* develop neonatal polycystic renal disease that progresses to renal failure (122).

Alternatively spliced isoforms of *bcl-x* protect from *(bcl-xL)* or predispose to *(bcl-xS)* apoptotic cell death in growth factor-deprived cells (123,124). The spectrum of the protection afforded by Bcl-xL against noxious stimuli overlaps with that of Bcl-2 (43,123). Bcl-xL protection may be more effective that Bcl-2, as in the case of cyclosporine A-induced apoptosis in lymphocytes (125). Enforced expression of Bcl-xL decreases the expression of endogenous Bcl-2 and vice versa (43). This observation of reciprocal regulation could explain the finding that antisense oligonucleotides for *bcl-2* do not alter mesangial cell susceptibility to apoptosis (118).

Murine kidneys and cultured renal cells express two *bcl-x* mRNA transcripts, detectable by Northern blot hybridization; the smaller one having a shorter half life (37). Both murine transcripts hybridize to a human *bcl-xL* specific probe, suggesting that *bcl-xL* is the main isoform in the kidney (37,126).

3.2.3.2. Regulatory factors that promote apoptosis

Some factors antagonize the protective effect of Bcl-2 and Bcl-xL, and accelerate cell death when the microenvironment is permissive for death. These proteins differ, however, from effectors, because they

do not induce cell death when the microenvironment supports survival.

Bax forms homodimers, as well as heterodimers with Bcl-2 and Bcl-xL (127,128). The BH1 and BH2 domains in Bcl-2 and Bcl-xL are required for the cell repressor activity and also for heterodimerization with Bax, but not for homodimerization (129). When optimal relative amounts of Bcl-2/Bax or Bcl-xL/Bax heterodimers are present, cells are protected from death induced by survival factor deprivation (127-129). If the percentage of free Bax is higher, cell death is more likely to occur (127-129).

It was originally reported that the murine kidney abundantly expresses two *bax* transcripts of 1.5 and 1 kb (127). We have found the 1 kb *bax* transcript to be preferentially expressed by mesangial cells, proximal tubular cells, metanephric stem cells as well as by the whole kidney (37). Bax immunoreactivity is readily detectable in renal tubular epithelial cells *in vivo* (130), where it appears to be more abundant than Bcl-2 (120,130).

Bad has homology to the BH1 and BH2 domains of Bcl-2 and has no lethal activity by itself (128). Bad heterodimerizes with Bcl-xL and Bcl-2, displaces Bax and reverses the death repressor activity (128). The **Bcl-xS** isoform of Bcl-x lacks the BH1 and BH2 domains and inhibits the ability of Bcl-2 to protect from cell death (123). **Bak** binds to Bcl-xL and antagonizes the protection offered by Bcl-2 when the cell is deprived of survival factors (131-133). Bak is present in the normal kidney (133).

3.2.3.3. Antiapoptotic regulatory factors

Bag-1 binds to Bcl-2 and enhances its survival promoting activity (115). The existence of Bag-1 or related factors may explain the conflicting reports on the ability of Bcl-2 to protect from Fas-induced apoptosis. Bcl-2 protection against this form of cell death is only complete when Bag-1 expression is high (115).

3.2.4 Other apoptosis related factors: clusterin

Clusterin is increased in acute and chronic renal failure associated with the occurrence of apoptosis (reviewed in 134). However, the relationship between clusterin and apoptosis is unclear (135-137). Clusterin inhibits the membrane attack complex of complement and is deposited in immune-mediated glomerular injury (134). In cultured mesangial cells, clusterin expression is regulated by cytokines (20).

4. APOPTOSIS IN THE KIDNEY

The occurrence of apoptosis has been demonstrated in several renal diseases. However, there are numerous unanswered questions regarding the precise role of apoptosis in renal damage and the extracellular and intracellular factors that induce and prevent apoptosis in the kidney. The possible therapeutic value of the modulation of renal cell apoptosis in kidney diseases has not been adequately investigated yet.

4.1 Role of apoptosis in renal disease

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

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

Apoptosis may play a role in the loss of parenchymal cells at several stages of renal damage. The correct knowledge of the contribution of apoptosis to each of these stages in different renal pathologies is required for the design of therapeutic strategies.

Apoptosis triggered by ischemia, exogenous toxins or endogenous mediators of damage may be the **initial insult** capable of causing renal disease. Apoptosis may also contribute to the **persistence** of renal injury. Thus, foci of inflammation in response to other stimuli may render the microenvironment inappropriate to cell survival. **Resolution** of hypercellularity in proliferative glomerulonephritis or during the recovery phase of acute renal failure may result from apoptosis of the redundant cells. **Progression** of renal disease may be a consequence of a persistently high apoptotic rate of renal parenchymal cells leading to glomerular or tubular atrophy. Alternatively, a low rate of fibroblast apoptosis may promote renal fibrosis.

4.1.2. 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 (138,139). Less frequently, a full blown inflammatory response that includes other leukocytes such as neutrophils and eosinophils is observed (140). Inflammatory cells may provide factors that cause parenchymal cell apoptosis. Monocytes/macrophages release TNF-alpha, Fas ligand, oxygen radical species, and nitric oxide (29,141). In contrast, the combination of perforin/granzyme B and Fas ligand accounts for most of the cytotoxicity of T lymphocytes (55).

In addition, apoptosis may stimulate or quench the inflammatory response. In the following sections the reciprocal interactions of apoptosis and inflammation are discussed.

4.1.2.1. Can apoptosis cause inflammation?

There is a harbored notion that apoptosis does not generate inflammation. However, generation of apoptosis and inflammation may be associated. Some

factors, such as TNF-alpha, induce both apoptosis and chemotactic factors that lead to the recruitment of leukocytes (20,21,139,142). For example, in tubular cells the dose response curve for TNF-alpha-induced *rantes* mRNA expression and apoptosis are similar (21,142). Apoptosis itself might promote inflammation through two mechanisms: 1) **Lysis of the apoptotic cells**. Disintegration of apoptotic cells with release of non-specific pro-inflammatory factors may be a consequence of a failure of the recognition/engulfing mechanism. The presence of a low pH, cationic molecules and fragments of extracellular matrix proteins interfere with the uptake of apoptotic cells by phagocytes (143-145). The reactivity of antiphospholipid antibodies with the phosphatidylserine exposed on the surface of apoptotic cells might also interfere with their clearance (146). Massive apoptosis occurring in an organ not physiologically prepared for such an event may also lead to failure of apoptotic cell clearance. For example, apoptotic cell lysis has been observed after Fas activation in the liver (52). 2) Active **release of proinflammatory cytokines**. The genetic program activated during apoptosis may provide specific chemotactic substances for phagocyte recruitment, as it does provide new surface determinants for recognition and phagocytosis. There is scattered evidence for this notion. ICE is a component of the apoptotic machinery and it also activates the proinflammatory cytokine IL-1ß (96,106). In fact, macrophages undergoing apoptosis, but not those undergoing necrosis, process IL-1ß (147). Cell recruitment may also depend on binding of apoptotic bodies to specific receptors on the surface of monocytes with resulting cell activation and cytokine release (148).

4.1.2.2. Regulation of inflammation by apoptosis.

Clearance of inflammatory cells by apoptosis may contribute to resolution of inflammation and failure of this clearance may contribute to the persistence of the inflammatory process. Leukocytes, including neutrophils, macrophages and lymphocytes undergo apoptosis if there is not an adequate amount of survival factors (149-152). This phenomenon has been observed *in vivo* in the resolution of renal inflammation (153,154). Apoptotic leukocytes may be engulfed by local cells, like mesangial cells (153). Survival factors for leukocytes may differ from those for renal cells. TNF-alpha, for example, prevents apoptosis in monocytes, but kills renal cells (20,21,151). Cytokines expressed by renal cells, such as TNF-alpha and Fas ligand (27,29), may contribute to the progression of renal disease through prolonged survival or activation of macrophages and T lymphocytes (58,151,152).

4.1.3 Apoptosis and the immune response

Apoptosis plays a fundamental role in the control of the immune response in the thymus and the periphery (12). While the details of this involvement are beyond the scope of this review, it should be noted that alterations in apoptosis-related genes such as *bcl-2* and *fas* result in autoimmune diseases and renal damage (53,60-63,121). Apoptosis by itself may generate autoimmunity. In effect, autoreactivity has been recognized against antigens present in apoptotic cells (155). If apoptotic cells are not adequately cleared, their contents might be released and further stimulate this autoimmune response (156). It has been suggested that this may help explain the relationship between infection and the initiation/exacerbation of autoimmunity, as infection can trigger apoptosis (4).

4.2. Expression of apoptosis genes in renal disease

In this section we will review information regarding the role of apoptosis and apoptosis genes in different forms of renal damage.

4.2.1 Glomerular injury

Cell turnover in the healthy glomerulus is low. Apoptotic cells represent about 0.01% of rat glomerular cells (22) and 0.03/10 glomerular cross-sections in humans (157). During renal injury apoptosis may contribute to the clearance of excessive intrinsic glomerular cells and leukocytes (158). The resolution of the mesangial proliferation characteristic of anti-Thy-1 nephritis depends on apoptosis of excessive mesangial cells, which peaks at 0.25% of glomerular cells (22). Apoptosis of neutrophils is prominent in nephrotoxic nephritis in the rat (140,153) and in acute postinfectious glomerulonephritis in humans (157). More recently, apoptosis has been noted in the first hours (<12h) after induction of anti-Thy1 nephritis (159,160). Together with the ability of anti-Thy1 to induce apoptosis of cultured mesangial cells (31,159), this suggests that apoptosis may also cause glomerular injury. An increased occurrence of apoptosis is also present in several experimental models of progressive glomerular scarring that include the nephropathy seen in growth hormone transgenic mice, adriamycin nephropathy, 5/6 nephrectomy and crescentic anti-glomerular basement membrane antibody-induced nephropathy (161,162). A 50 to 100-fold increase in apoptosis was observed in human proliferative nephritis such as IgA nephropathy, lupus nephritis and anti-neutrophil cytoplasm antibody (ANCA)-positive vasculitis (157,163). The role of apoptosis in these diseases is unclear. By contrast the rate of apoptosis was much lower in non-proliferative glomerulonephritis such as membranous nephropathy (159,163). Apoptosis of glomerular epithelial and endothelial cells has also been noted during glomerular injury.

Glomerular apoptosis appears to be related to changes in the local expression of apoptosis regulatory genes. Expression of lethal factors such as TNF-alpha and *fas ligand* is increased in several types of glomerular injury (29 and reviewed in 164). Increased expression of *fas*, *bcl-2* and *bax* has been observed in mesangial cells in proliferative glomerulonephritis (65,165,166). An association was found between

glomerular Fas expression and glomerular cell death (166). The Bcl-2/*bax* ratio decreased in glomeruli showing matrix expansion with decreased cellularity (165). Bcl-2 deficient mice display marked glomerular abnormalities (122) that resemble the epithelial crescents typical of rapidly progressive glomerulonephritis. Bcl-2 and *bax* expression was not different from controls in patients with diseases characterized by glomerular normocellularity such as minimal change nephrotic syndrome and membranous nephropathy (165). High ICE mRNA levels are present in the experimental models of progressive glomerular scarring mentioned above (161).

4.2.2 Acute renal failure

Tubular cell apoptosis has been observed during ischemic, toxic and obstructive acute renal failure (15,17,167-171). In these conditions there is also an on-going necrosis and the relative contribution of the two mechanisms of death to the initial cell loss is uncertain. Apoptosis may also occur in cells proliferating in a compensatory fashion after renal injury. These cells may be more sensitive to absolute or relative deficits in survival factors. In this setting apoptosis might contribute to the persistence or delayed recovery from acute renal failure. Apoptosis may also contribute to an adequate resolution of damage (168,170,171). In this case it may represent a physiologic balance to check an exaggerated compensatory proliferative response. Shimizu *et al.* observed, after 60 minutes of ischemia, an early peak of necrosis and apoptosis in the first 48-72h of acute renal failure, and a second, bigger peak of apoptosis after 7-14 days, when the necrotic tubules had been completely reconstituted by a hyperplastic epithelium (168).

Changes in the expression of both extracellular and intracellular apoptosis regulatory factors occur during acute renal failure. A cytokine microenvironment permissive for cell death includes decreased renal levels of *pre-pro-EGF*, *IGF-1* and *TGF-alpha* mRNA (169,172-174), and increased systemic TNF-alpha and local TGF-ß1 and *fas ligand* (174,175 and unpublished observation). Among the receptors, renal Fas is increased in experimental septic and toxic acute renal failure (65,176), as well as in human acute tubular necrosis (176). Expression of transcription factors involved in apoptosis is also high. *c-myc* and c-*fos* are increased in the early stages of several models of acute renal failure (177,178). c-myc overexpression confers a dependence on external survival factors (75), and it may promote cell death in the adverse cytokine microenvironment found in acute renal failure. c-myc activates p53 transcription (78). More recently, increased p53 expression has also been noted in obstructive nephropathy (179). This finding is in agreement with our prior observation that *bcl-2* is decreased and *bax* is increased during toxic acute renal failure in mice (37). Another apoptosis regulatory gene, *bcl-xL* is also increased in this model (37). Bcl-2 is decreased in obstructive nephropathy (180).

4.2.3 Chronic renal atrophy and renal fibrosis

Chronic renal atrophy is characterized by a progressive loss of renal parenchymal cells (181). Apoptosis of tubular epithelial cells has been observed in chronic tubular atrophy induced by chronic ischemia, papillary necrosis, subtotal nephrectomy and HIV nephropathy (15,17,182,183). The interstitial infiltration by macrophages and T cells in progressive kidney diseases may provide cytokines and inflammatory mediators that induce apoptosis (138,139).

Parenchymal atrophy and interstitial fibrosis are almost invariably associated. However, the relationship between these two phenomena is poorly understood. Transdifferentiation of parenchymal cells (such as tubular epithelial cells) into fibroblasts may explain their association (184). Alternatively, fibrosis may promote atrophy by providing an adverse microenvironment for epithelial cell survival. Both an abnormal extracellular matrix (25,26) and the release of cytokines that induce apoptosis, such as TNF-alpha and Fas ligand, by fibroblasts (29) may contribute to this microenvironment.

Accumulation of interstitial fibroblasts may also be caused by altered regulation of cell survival. Fibroblasts obtained from fibrotic kidneys accumulate more rapidly in culture and survive longer than those obtained from healthy kidneys (185). In this sense, fibroblasts involved in skin wound repair are eliminated by apoptosis (186).

4.2.4 Polycystic renal disease

Both the occurrence of apoptosis and abnormalities of apoptosis-related genes have been reported in polycystic kidneys (187-189). Transgenic mice overexpressing *c-myc* suffer from polycystic kidneys (188), and overexpression of *c-myc* has been detected in the kidneys of *cpk/cpk* mice (189). The lack of functional Bcl-2 results in murine neonatal polycystic renal disease (122). It is theoretically possible that overexpression of *bax*, the endogenous antagonist of *bcl-2*, may result in the development of renal cysts. However, levels of *bax* mRNA in kidneys or tubular cells from *cpk/cpk* mice were normal (unpublished observation).

The PKD1 gene, responsible for most of the cases of human autosomal dominant polycystic kidney disease has recently been cloned (190). The encoded protein may participate in interactions with extracellular ligands. The intriguing possibility that it may mediate cell survival or death has not been addressed yet.

4.2.5 Kidney development

Apoptosis decreases the mass of unneeded metanephric mesenchyme following induction by the ureteric bud (14,191). NGF, EGF, IGF-I, IGF-II, TGFalpha and HGF can rescue metanephric cells from apoptosis (14,192,193). Metanephric stem cells also possess receptors for lethal factors such as Fas and TNF-alpha (unpublished observation).

Developing kidneys express high levels of *bcl-2*, *bak*, *FADD*, *bcl-xL*, *ich-1L* and *p53* (74,102,119,126,133,194). Our own data indicate that Bcl-2, *bax* and *bcl-xL* are expressed by metanephric stem cells in culture (195 and unpublished). However, as already suggested by the normal renal phenotype of ICE-deficient mice (98), ICE mRNA was not detected in the developing kidney (161).

Altered apoptosis during kidney development can result in renal dysplasia or agenesis. Mice carrying targeted mutations of *bcl-2* have small neonatal polycystic kidneys with persistence of immature cells (122,196). Mice lacking functional WT-1 suffer from renal agenesis as a result of massive apoptosis of the metanephric blastema (197).

5. PERSPECTIVES FOR MODULATION OF APOPTOSIS IN THE THERAPY OF RENAL DISEASES

Understanding the role and regulation of apoptosis in renal disease may improve our knowledge of the mode of action of current therapies, and it may also provide the basis for the design of new therapeutic strategies. These newer therapies may include drugs that interfere with apoptosis, antisense strategies, local delivery of protective genes, and antagonism of cytokines or lipid mediators. Although the precise timing and potential cellular and genetic targets of apoptosis modulatory therapies remain to be defined, evidence is accumulating that it may be beneficial to interfere with apoptosis in renal disease. In this sense, two of the most widely used drugs for the therapy of renal transplant recipients and patients with glomerulonephritis, corticosteroids and cyclosporine A, promote lymphocyte apoptosis (36,125).

Among the cellular targets, we might be interested in prolonging parenchymal cell survival in chronic renal atrophy. Identification of the survival signals for leukocytes may help to selectively manipulate their survival without a deleterious effect on renal cells, in order to resolve inflammation. Identification of cell-specific promoters may facilitate targeting of genes that promote apoptosis to fibroblasts to treat renal fibrosis (198).

Tools employed to influence apoptosis may include cytokines and cytokine antagonists, which have already been used in experimental renal disease. Therapy with EGF improves the evolution of experimental acute renal failure and decreases internucleosomal DNA degradation (199,200). The administration of IGF-I to Bcl-2+/- pregnant mice resulted in a 49% increase in the weight of kidneys of Bcl-2-/- offsprings (193). This may be a basis for intrauterine therapy of renal dysplasia. Antagonism of TNF-alpha improves the evolution of experimental models of acute renal failure and several glomerulonephritis (reviewed in 164). Antagonism of TGF-ß$_1$ has also been shown to be protective (201). Although both cytokines may induce apoptosis, the contribution of apoptosis blockage to the therapeutic effect of their antagonists has not been addressed.

Extracellular matrix-cell interactions might also be targeted in the therapy of renal diseases. In cancer therapy blockage of integrin receptors leads to apoptosis of new vessels and subsequent tumor regression (202). In nephrology it is unclear the possible relationship between the beneficial effect of therapies involving extracellular matrix components such as fibronectin (138) with their ability to interfere with apoptosis.

Specific targeting of proteins carrying the death domain, Bcl-2 like proteins and apoptosis proteases may also be of value in the therapy of renal diseases.

Another approach is to improve disposal of the apoptotic cells, preventing their lysis. For example, CD36 gene transfer confers the capacity to cells to ingest apoptotic cells if the vitronectin receptor is also expressed and thrombospondin is available (203).

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