

Role of Caspases in Renal Tubular Epithelial Cell Injury

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The regulation of cell death has been investigated in a number of clinical disorders including renal ischemic and toxic acute renal failure. Caspases play a crucial role in the execution or final phase of cell death by cleaving and inactivating various structural and functional intracellular proteins that are essential for cell survival and proliferation. Evidence is now emerging to implicate the caspase pathway in a variety of renal diseases including the pathogenesis of acute renal failure. Among the 14 known members of the caspase family thus far identified several executioner caspases including caspases-3, -6, and -7 and the proinflammatory caspase including caspase-1 may participate in the final degradation of intracellular proteins. The activation of these caspases is regulated by the receptor- and mitochondrial-mediated cell signaling pathways as well as by the endoplasmic reticulum stress response. While the role of some caspases in renal injury is emerging, the roles of various proinflammatory and other executioner caspases remain to be determined. Although many pro- and anti-apoptotic molecules that act upstream of caspase activation have been identified, their regulation is yet to be determined in the pathogenesis of renal injury. A precise description of caspase-mediated cell death pathway and regulation of caspase activation is, therefore, critical to the understanding of the mechanism of renal injury and to the development of therapeutic targets that prevent renal diseases and preserve renal function.

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CONSIDERABLE EVIDENCE is now accumulating to implicate apoptosis and the caspase pathway in the pathophysiology of acute renal failure. Caspases are a family of cell death proteases¹ that play an essential role in the execution of apoptosis.²⁻⁷ They cleave and inactivate various structural and functional intracellular proteins that are essential for cell survival and proliferation. This cleavage of target proteins contributes to the morphologic changes observed in apoptosis. The term *caspase* for the cell death proteases embodies 2 distinct catalytic properties of these enzymes such that “c” refers to the cysteine protease and “aspase” refers to their specific ability to cleave after an aspartic acid. The role of caspases in apoptosis was first recognized in 1993⁸ when it was discovered that the cell death gene CED3 in *Caenorhabditis elegans* had sequence homology to caspase-1, which was then called interleukin-1 β (IL-1 β)-converting enzyme.⁸

At least 14 caspases encoded by distinct genes have been identified to date from mammalian cells^{2-4,9,10}; they are divided into 2 main subfamilies based on sequence homology to caspase-1 and CED 3. Caspases-8, -10, -2, and -9 have larger prodomains and are termed *initiator caspases*, whereas caspases with smaller domains such as caspases-3, -7, and -6, are termed *executioner caspases*. In general the initiator caspases are responsible for the activation of downstream executioner caspases. Caspases-1, -4, -5, -11, and -13 play a role in inflammation.^{3,4,11} Caspase-12 is an endoplasmic reticulum-associated protease that is activated in response to endoplasmic reticulum

stress¹² and is involved in the endoplasmic reticulum stress-induced apoptosis.^{12,13} Overexpression of executioner and initiator caspases in transfected cells results in DNA fragmentation and cell death in a variety of mammalian cell lines.^{11,12,14,15} Caspases share many common features such as: (1) they are synthesized as inactive proenzymes mostly in the cytosol of living cells. Each proenzyme is composed of 3 structural domains: a variable prodomain, a large subunit of about 20-kd size, and a small subunit of about 10-kd size. On receiving an apoptotic stimulus, these domains are cleaved proteolytically and the large and small subunits oligomerize to form an active enzyme.^{2,15} (2) Most of the caspases are capable of initiating an apoptotic response when transfected into recipient cells.^{3-5,7,16} (3) The caspases are inhibited by substrate-specific synthetic peptide inhibitors and by the baculovirus protein, p35,¹⁷ or by the poxvirus serpin, CrmA.¹⁸ In cell culture, these inhibitors suppress mammalian cell apoptosis. (4) Caspases are very specific proteases with an absolute requirement for cleavage after aspartic acid in the target substrates. They require a tetrapeptide rec-

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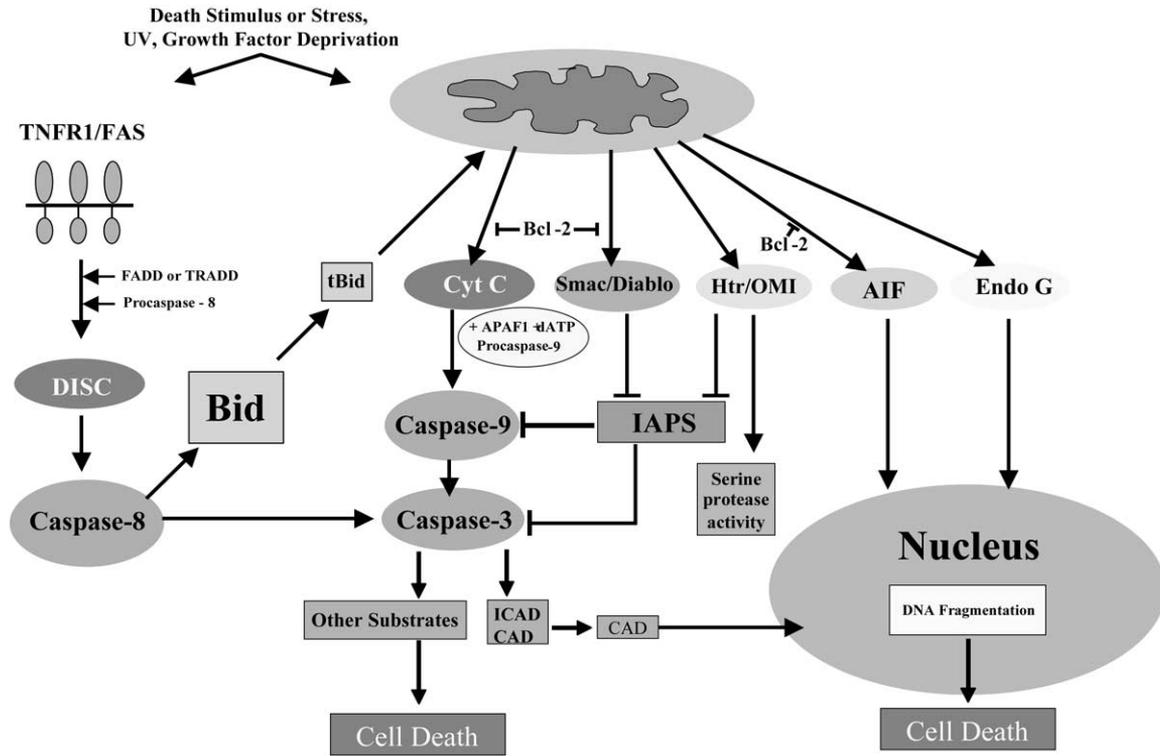


Fig 1. Receptor- and mitochondrial-mediated pathways of cell death and caspase activation.

ognition sequence with c-terminus aspartate for cleavage in target proteins. (5) The active site contains the sequence QACxG in which C is a catalytic cysteine.^{2,7,9,12}

At present, there are 2 relatively well-characterized cell death pathways that result in the activation of executioner caspases (Fig 1). One is receptor mediated and the other is mitochondrial dependent. On receiving an apoptotic stimulus, the receptor-dependent pathway is initiated by activation of cell death receptors such as Fas/CD95/APO1 and tumor necrosis factor (TNF)-R1. The homophilic interactions between the conserved death domains of the receptor and the adaptor molecule such as FAS associated death domain protein (FADD) or TNF receptor associated death domain protein (TRADD) form receptor-adaptor complexes that recruits the initiator caspase, procaspase-8, resulting in the formation of a death-inducing signaling complex. The death-inducing signaling complex then facilitates the proteolytic processing of procaspase-8 to its active form. Caspase-8 in turn cleaves and activates downstream executioner caspases, caspases-3, -6, and

-7.¹⁹⁻²¹ Despite wide expression, Fas death signals frequently are interrupted. A cytosolic protein, FLIP (Fas-associated death domain like IL-1 β -converting enzyme-inhibitory proteins), which structurally resembles caspase-8, interferes with the activation of procaspase-8 by binding to FADD and results in the failure to activate the downstream caspases.^{22,23} Receptor-induced cell death and caspase-8 activation also is inhibited by the cowpox virus protein Crm A²⁴⁻²⁶ but not by Bcl-2.²⁷ However, inhibition by Fas-associated death domain-inhibitory proteins suggests their critical role as endogenous modulators of apoptosis.^{22,23}

The other pathway of caspase activation is mitochondrial dependent and is triggered by cytochrome c release from the mitochondria. In the cytosol, cytochrome c recruits a cytosolic protein Apaf-1, allowing Apaf-1 to bind to the nucleotide deoxyadenosine triphosphate. The binding of deoxyadenosine triphosphate to the Apaf-1-cytochrome c complex induces its oligomerization to form a high molecular weight caspase-activating complex termed the *apoptosome*. The apoptosome recruits procaspase-9, which facilitates proteolytic

processing of procaspase-9 to its active form. Activated caspase-9 then cleaves and activates downstream pro-caspase-3.²⁸⁻³⁰ An active site mutant of caspase-9 is able to block activation of caspase-3 by caspase-9.²⁹ Recent studies have documented that during apoptosis other proteins including the second mitochondrial activator of caspases (Smac) or the direct inhibitors of apoptosis proteins (IAPs) binding protein with low pI (Diablo), apoptosis-inducing factor, endonuclease G, and serine protease HtrA2/Omi also are released from the mitochondria along with the release of cytochrome c (Fig 1). Smac/Diablo, once released from mitochondria, are involved in the regulation of caspase activation by binding to IAPs that are known to inhibit caspases.^{31,32} IAPs are characterized by the presence of one or more baculovirus IAP repeat domains. X chromosome-encoded IAP (XIAP), an active member of the IAP family, has been shown to directly inhibit caspases-3, -7, and -9³³⁻³⁵ through an interaction involving baculovirus IAP repeat domains. Thus, Smac/Diablo promotes cell death by binding to IAPs that liberate caspases from IAP inhibition or by preventing IAP binding to active caspases. Apoptosis-inducing factor, once released from the mitochondria, induces cell death independent of activation of caspases. In response to an apoptotic stimulus, apoptosis-inducing factor is translocated to the cytosol and to the nucleus where it induces DNA fragmentation with high molecular weight fragments compared with much smaller DNA fragments obtained by caspase-activated deoxyribonuclease. Overexpression of anti-apoptotic Bcl-2 family members, bcl-2 or bclL, blocks apoptosis-induced mitochondrial release of cytochrome c,³⁶⁻³⁸ Smac/Diablo,^{39,40} and apoptosis-inducing factor.^{41,42} Thus, Bcl-2 is an important gatekeeper of mitochondria that plays an effective role in controlling the release of important pro-apoptotic molecules from the mitochondria.

IN VITRO EVIDENCE OF CASPASE ACTIVATION IN CYTOTOXICITY

In studies in vitro, caspases are involved in hypoxic^{43,44} injury to renal tubular epithelial cells. Antimycin A-induced chemical hypoxia⁴³ or growth under hypoxic conditions results in increased caspase activity and pancaspase inhibition prevents hypoxia-induced DNA fragmentation and cell death in renal tubular epithelial cells. Partial adenosine triphosphate depletion of MDCK cells

by antimycin A also was shown to result in apoptosis with marked increase in activation of caspase-8 and inhibition of caspases provided marked protection against antimycin A-induced cell death.⁴⁵ Exposure of freshly isolated renal tubular epithelial cells to hypoxia resulted in caspase activation and cell membrane damage.⁴⁴ In a related study, activation of caspase-3 during hypoxia or adenosine triphosphate depletion was accompanied by Bax translocation and cytochrome c release.⁴⁶ Thus, these studies in in vitro models of ischemic injury suggest that caspases may play important roles in ischemic acute renal failure. As in ischemia, nephrotoxic agents such as cisplatin also activate the caspase cascade as well. Cisplatin-induced cell death in renal tubular epithelial cells is accompanied by the activation of caspase-3.⁴⁷⁻⁴⁹ Recent studies in renal tubular epithelial cells showed that cisplatin induced selective and differential activation of caspases including executioner caspase-3 and initiator caspases-8, -9, and -2, but not proinflammatory caspase-1.⁵⁰ The translocation of Bax to the mitochondria and release of cytochrome c from the mitochondria to the cytosol further support the activation of caspase-9.⁵¹ Cisplatin-induced activation of caspase-8 also has been reported in freshly isolated proximal tubular epithelial cells.⁵² The activation of cisplatin-induced caspases was markedly inhibited by their respective peptide inhibitors, suggesting that these caspases may play an important role in cisplatin-induced injury to renal tubular epithelial cells. DEVD-CHO or LEHD-CHO, inhibitors of caspases-3 and -9, respectively, provided partial protection against cisplatin-induced cell death and DNA damage in LLC-PK1 cells,⁵⁰ indicating mechanisms other than caspase activation also are involved in cisplatin-induced cell death. Overexpression of crmA, a cowpox viral gene known to inhibit caspase-8, also provided protection against cisplatin-induced apoptosis in mouse proximal tubular cells.⁴⁸ In fact, cisplatin induced increased expression of TNF- α and Fas,⁵² which typically is associated with caspase-8 activation, and was capable of inducing apoptosis in renal epithelial cells. Thus, cisplatin-induced activation of caspases-8 and -9 in renal proximal tubules indicated that both receptor and mitochondrial signaling pathways participate in the activation process. In a recent study, p53 inhibition was shown to partially protect cisplatin-induced cell death,⁵³ indicating that p53

may also be involved in cisplatin-induced activation of caspases in renal tubular epithelial cells.

CASPASES IN KIDNEY AND IN VIVO EVIDENCE OF CASPASE ACTIVATION IN CYTOTOXICITY

Most of the members of the caspase family are expressed in the kidney.^{54,55} However, there are limited studies on the localization of caspases in the kidney. Caspases-3 and -6 are localized predominantly in renal tubular epithelium^{55,56} and caspases-1 and -2 were localized within murine fetal kidney. The precise localization of the various caspases in different segments of the kidney is not yet known.

Rat kidneys subjected to ischemia-reperfusion injury revealed differential expression of caspases with marked increase in caspase-1 and -3 messenger RNA (mRNA) and proteins during the early period (4-16 h after ischemia) of reperfusion injury. A transient increase in caspase-2 mRNA and protein has been observed during ischemia and the early period of reperfusion. Only minimal changes in caspase-6 mRNA expression have been observed during the ischemia or reperfusion period.⁵⁴

The proforms of caspase-1 and -3 proteins were cleaved to their active forms during reperfusion injury, indicating activation of these enzymes.⁵⁴ A significant increase in caspase-1 and -3 activities were reported in a murine model of renal ischemia-reperfusion injury.⁵⁷ The administration of a pan-caspase inhibitor, Z-VAD-FMK, at the time of reperfusion significantly prevented caspase-1 and -3 activities, and provided marked protection not only against reperfusion-induced DNA damage (as determined by TUNEL assay), and subsequent inflammation, but also ischemic acute renal failure.⁵⁷ These results in renal ischemic injury seem consistent with the study performed on ischemic injury to gerbil forebrain⁵⁸ and rat brain.⁵⁹ A study on global forebrain ischemia reported increased mRNA and protein expression of caspase-1 at 48 hours after ischemia in gerbils.⁶⁰ Increased induction of caspase-3 mRNA at 16 hours through 24 hours after ischemic injury also has been reported in rat brain after permanent occlusion of the middle cerebral artery.⁵⁹

The specific role of proinflammatory caspase-1 recently has been examined in ischemic acute renal failure. Caspase-1 is involved in the proteolytic cleavage of the precursor forms of proinflammatory

cytokines IL-1 β and IL-18 that result in the formation of active forms of mature cytokines.^{61,62} Because caspase-1-mediated formation of active IL-1 β ⁶¹ and IL-18⁶² are associated with inflammatory response in renal ischemia-reperfusion, caspase-1 may play an important role in ischemia-reperfusion injury. Thus far, 2 studies have investigated the role of caspase-1 in ischemia-reperfusion injury using caspase-1 (-/-) mice but the results remain somewhat inconsistent. One study reported that caspase-1 (-/-) mice afforded significant protection against ischemia-reperfusion as reflected by renal function and renal histology,⁶³ whereas the other study showed that caspase-1 (-/-) mice did not provide protection against ischemia-reperfusion as revealed by renal function with no change in blood urea nitrogen and serum creatinine levels.⁶⁴ However, a recent study showed that caspase inhibitor Quinoline-Val-Asp(Ome)-CH₂-OPH (OPH-001) markedly decreased ischemia-reperfusion-induced caspase-1 activity and IL-18 protein, prevented neutrophil infiltration, and attenuated acute tubular necrosis (ATN) as reflected in marked reduction in blood urea nitrogen and creatinine levels.⁶⁵ Although this inhibitor blocked caspase-1 activity, the specificity of this inhibitor toward other caspases in this study is not precisely known. These studies further explored the role of neutrophil infiltration in increased IL-18 production in ATN using neutrophil-depleted mice. Caspase-1 and IL-18 proteins in neutrophil-depleted mice were increased significantly and neutralizing antibody to IL-18 provided significant protection against ATN as reflected by a 75% reduction in serum creatinine level and a significant reduction in the ATN score compared with control. Although these studies suggest a neutrophil-independent mechanism of IL-18-mediated ischemic acute renal failure,⁶⁵ more studies are required to show the definitive contribution of caspase-dependent and caspase-independent formation of inflammatory products for the induction of inflammation and apoptosis in ischemic acute renal failure. Caspase-3 activation during ischemia-reperfusion injury also may be involved in the down-regulation of calpastatin, an inhibitor of calpain,⁶⁶ indicating a role of caspases for calpain activation during renal injury.

REGULATION OF CASPASE ACTIVATION IN RENAL TUBULAR INJURY

There is little information on the mechanisms underlying the activation of caspases in renal in-

jury. The death receptor-mediated pathway that activates caspase-8 and subsequently executioner caspases may play a vital role in renal tubular cell death in ischemia reperfusion,⁶⁷ cisplatin-induced nephrotoxicity,⁵² and chronic renal failure.⁶⁸ Renal tubular epithelial cell apoptosis was decreased significantly when *lpr/lpr* C57BL/6 mice that express low levels of Fas were subjected to in ischemia-reperfusion injury⁶⁷ or cisplatin nephrotoxicity.⁵² Similar to Fas- FasL, the TNF- α -mediated death receptor pathway may play a vital role in acute renal ischemic,^{69,70} cisplatin,⁷¹ and endotoxemic⁷² injury as well. Inhibition of TNF- α action, either with a TNF binding protein⁶⁹ or neutralizing antibodies,⁷⁰ reduced ischemic injury in rats. Increased expression of Fas-FasL and TNF-R1, their associated adaptor molecules, and caspase-8 was observed in chronic obstructive nephropathy, suggesting that receptor-mediated pathway may play a role in this injury.⁷³ Thus, these studies suggest the role of activation of caspase-8 is associated with a death receptor-mediated pathway in tubular cell apoptosis.

The mitochondrial-dependent pathway that involves the release of cytochrome *c* and Smac/Diablo regulates the activation of caspase-9 and, subsequently, caspase-3. This signaling pathway is regulated by the members of the Bcl-2 family. The cell survival protein Bcl-2 has multiple anti-apoptotic effects including inhibition of the release of cytochrome *c*^{27,28,38} and Smac/Diablo^{39,40} from the mitochondria as well as binding to Apaf1 and preventing activation of caspase 9. In ischemia-reperfusion injury to the kidney the expression of both anti-apoptotic Bcl-2 and proapoptotic Bax has been reported.^{74,75} Because pro- and anti-apoptotic members of the Bcl-2 family heterodimerize with each other, the relative concentration of the proapoptotic and pro-survival members may act as a rheostat for cell death.⁷⁶

In some cells (type 1), the receptor-mediated pathway is linked to the mitochondrial-dependent caspase activation and cell death. These cells produce a high level of active caspase-8 that cleaves Bid, a member of the Bcl-2 family. The truncated Bid translocates from the cytosol to the mitochondria and induces the release of cytochrome *c*^{77,78} and subsequent activation of caspase-9. However, the interaction between the receptor-mediated and the mitochondrial-dependent pathways has yet to be determined in renal injury.

SUMMARY

Recent studies have documented that apoptosis and the caspase pathway play a vital role in the pathophysiology of acute renal failure. The role of caspases has been investigated in both in vitro and in vivo models of acute renal failure using broad-spectrum caspase inhibitors. Although caspases inhibitors have provided some success in ameliorating experimental ischemic acute renal failure, the precise identity of the caspases targeted by these inhibitors is not known. It is not known if broad-spectrum inhibitors of caspases are effective in other models of acute renal failure. Thus, more studies using more specific inhibitors are required to investigate their effect in different models of acute renal failure. Among the 14 known members of the caspases, caspases-3, -6, and -7 are the executioner caspases that are the major active caspases detected in apoptotic cells and are widely regarded to mediate the execution of apoptosis by cleaving and inactivating intracellular proteins that are essential for cell survival and proliferation. Future studies are needed to understand not only the precise identity of caspases involved but also their specific downstream substrates cleaved in acute renal failure. In addition, the proinflammatory caspases such as caspases-1, -4, and -5 may participate in the inflammatory processes in renal injury. Although studies have begun to examine the role of caspase-1, it is not known whether both inflammatory and executioner caspases together play a role in renal injury. Caspase knockout mice for the executioner and the inflammatory caspases will provide useful information whether these caspases play a role in renal tubular epithelial cell injury. At present, the molecular mechanisms that trigger caspase activation in renal injury are not precisely known. Also, there is little information on the pro- and anti-apoptotic molecules as well as the upstream signals that may regulate caspase activation in kidney in response to injury. Thus, future studies will address precise identification of caspases and the mechanisms involved in the regulation of their activation in renal tubular epithelial cell injury. This information is critical to the understanding of the mechanism of renal injury and to the development of specific therapeutic targets that prevent renal diseases.

REFERENCES

1. Alnemri E, Livingston D, Nicholson D, et al: Human ICE/CED-3 protease nomenclature. *Cell* 87:171, 1996
2. Thornberry N, Lazebnik Y: Caspases: Enemies within. *Science* 281:1312-1316, 1998
3. Fraser A, Evan G: A license to kill. *Cell* 85:781-786, 1996
4. Earnshaw W, Martins L, Kaufmann S: Mammalian caspases: Structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68:383-424, 1999
5. Cryns V, Yuan J: Proteases to die for. *Genes Dev* 12:1551-1570, 1998
6. Vaux D: Caspases and apoptosis-biology and terminology. *Cell Death Differ* 6:493-494, 1999
7. Nicholson D: Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Differ* 6:1028-1042, 1999
8. Yuan J, Shaham S, Ledoux S, et al: The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 β converting enzyme. *Cell* 75:641-652, 1993
9. Wolf B, Green D: Suicidal tendencies: Apoptotic cell death by caspase family proteinases. *J Biol Chem* 274:20049-20052, 1999
10. Ahmad M, Srinivasula S, Hegde R, et al: Identification and characterization of murine caspase 14, a new member of the caspase family. *Cancer Res* 58:5201-5205, 1998
11. Salvesen G, Dixit V: Caspases: Intracellular signaling by proteolysis. *Cell* 91:443-446, 1997
12. Nakagawa T, Zhu H, Morishima N, et al: Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid- β . *Nature* 403:98-103, 2000
13. Rao RV, Castro-Obregon S, Frankowski H, et al: Coupling endoplasmic reticulum stress to the cell death program. An Apaf-1-independent intrinsic pathway. *J Biol Chem* 277:21836-21842, 2002
14. Cohen G: Caspases: The executioners of apoptosis. *Biochem J* 326:1-16, 1997
15. Nicholson D, Thornberry N: Caspases. Killer proteases. *Trends Biochem Sci* 257:299-306, 1997
16. Nagata S: Apoptosis by death factor. *Cell* 88:355-365, 1997
17. Stennicke HR, Ryan CA, Salvesen GS: Reprieve from execution: The molecular basis of caspase inhibition. *Trends Biochem Sci* 27:94-100, 2002
18. Zhou Q, Snipas S, Orth K, et al: Target protease specificity of the viral serpin CrmA. Analysis of five caspases. *J Biol Chem* 272:7797-7800, 1997
19. Boldin M, Goncharov T, Goltsev Y, et al: Involvement of Mach, a novel MORT1/FADD-interacting protease, in Fas/APO-1 and TNF receptor-induced cell death. *Cell* 85:803-815, 1996
20. Kischkel F, Hellbardt S, Behrmann I, et al: Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 14:5579-5588, 1995
21. Ashkenazi A, Dixit VM: Death receptors: Signaling and modulation. *Science* 281:1305-1308, 1998
22. Krueger A, Baumann S, Kramer PH, et al: FLICE-inhibitory proteins: Regulators of death receptor-mediated apoptosis. *Mol Cell Biol* 21:8247-8254, 2001
23. Tschopp J, Irmeler M, Thome M: Inhibition of fas death signals by FLIPs. *Curr Opin Immunol* 10:552-558, 1998
24. Takahashi A, Hirata H, Yonehara S, et al: Affinity labeling displays the stepwise activation of ICE-related proteases by Fas, staurosporine, and CrmA-sensitive caspase-8. *Oncogene* 14:2741-2752, 1997
25. Tewari M, Dixit V: Fas and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product. *J Biol Chem* 270:3255-3260, 1995
26. Miura M, Friedlander R, Yuan J: Tumor necrosis factor-induced apoptosis is mediated by a CrmA-sensitive cell death pathway. *Proc Natl Acad Sci U S A* 92:8318-8322, 1995
27. Newton K, Strasser A: The Bcl-2 family and cell death regulation. *Curr Opin Genet Dev* 8:68-75, 1998
28. Green D, Reed J: Mitochondria and apoptosis. *Science* 281:1309-1312, 1998
29. Li P, Nijhawan D, Budihardjo I, et al: Cytochrome c and dATP-dependent formation of apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 91:479-489, 1997
30. Zou H, Henzel W, Liu X, et al: Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 90:405-413, 1997
31. Shi Y: Mechanisms of caspase activation and inhibition during apoptosis. *Mol Cell* 9:459-570, 2002
32. Ravagnan L, Roumier T, Kroemer G: Mitochondria, the killer organelles and their weapons. *J Cell Physiol* 192:131-137, 2002
33. Huang Q, Deveraux QL, Maeda S, et al: Evolutionary conservation of apoptosis mechanisms: Lepidopteron and baculoviral inhibitor of apoptosis proteins are inhibitors of mammalian caspase-9. *Proc Natl Acad Sci U S A* 97:1427-1432, 2000
34. Deveraux QL, Stennicke HR, Salvesen GS, et al: Endogenous inhibitors of caspases. *J Clin Immunol* 19:388-398, 1999
35. Takahashi R, Deveraux Q, Tamm I, et al: A single BIR domain of XIAP sufficient for inhibiting caspases. *J Biol Chem* 273:7787-7790, 1998
36. Lemasters J, Nieminen A, Qian T, et al: The mitochondrial permeability transition in toxic, hypoxic and reperfusion injury. *Mol Cell Biochem* 174:159-165, 1997
37. Vander Heiden M, Chandel N, Williamson E, et al: Bcl-x_L regulate the membrane potential and volume homeostasis of mitochondria. *Cell* 91:627-637, 1997
38. Zamzami N, Marchetti P, Castedo M, et al: Inhibitors of permeability transition interfere with the disruption of the mitochondrial transmembrane potential during apoptosis. *FEBS Lett* 384:53-57, 1996 (abstr)
39. Sun X-M, Bratton SB, Butterworth M, et al: Bcl-2 and Bcl-x_L Inhibit CD95-mediated apoptosis by preventing mitochondrial release of Smac/DIABLO and subsequent inactivation of x-linked inhibitor-of-apoptosis protein. *J Biol Chem* 277:11345-11351, 2002
40. Adrain C, Creagh EM, Martin SJ: Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. *EMBO J* 20:6627-6636, 2001
41. Susin SA, Lorenzo HK, Zamzami N, et al: Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397:441-446, 1999
42. Susin SA, Zamzami N, Castedo M, et al: Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J Exp Med* 184:1331-1341, 1996
43. Kaushal G, Ueda N, Shah S: Role of caspases (ICE/CED

- 3 proteases) in DNA damage and cell death in hypoxic injury. *Kidney Int* 52:438-445, 1997
44. Edelstein C, Shi Y, Schrier R: Role of caspases in hypoxia-induced necrosis of rat renal proximal tubules. *J Am Soc Nephrol* 10:1940-1949, 1999
45. Feldenberg L, Thevananther S, Del Rio M, et al: Partial ATP depletion induces Eas-and caspase-mediated apoptosis in MDCK cells. *Am J Physiol* 276:F837-F846, 1999
46. Saikumar P, Dong Z, Patel Y, et al: Role of hypoxia-induced Bax translocation and cytochrome c release in reoxygenation injury. *Oncogene* 17:3401-3415, 1998
47. Lieberthal W, Triaca V, Levine J: Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: Apoptosis vs. necrosis. *Am J Physiol* 270:F700-F708, 1996
48. Takeda M, Kobayashi M, Shirato I, et al: Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1beta converting enzyme (ICE) family of proteases but inhibited by overexpression of Bcl-2. *Arch Toxicol* 71:612-621, 1997
49. Lau AH: Apoptosis induced by cisplatin nephrotoxic injury. *Kidney Int* 56:1295-1298, 1999
50. Kaushal G, Kaushal V, Hong X, et al: Role of caspases and their regulation by Akt/protein kinase B phosphorylation pathway in cisplatin-induced injury to renal tubular epithelial cells. *Kidney Int* 60:1726-1736, 2001
51. Park MS, De Leon M, Devarajan P: Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. *J Am Soc Nephrol* 13:858-865, 2002
52. Tsuruya K, Ninomiya T, Tokumoto M, et al: Direct involvement of the receptor-mediated apoptotic pathways in cisplatin-induced renal tubular cell death. *Kidney Int* 63:72-82, 2003
53. Cummings B, Schnellmann R: Cisplatin-induced renal cell apoptosis: Caspase 3-dependent and -independent pathways. *J Pharmacol Exp Ther* 302:8-17, 2002
54. Kaushal G, Singh A, Shah S: Identification of caspase (ICE-like proteases) gene family in rat kidney and altered expression in ischemia/reperfusion injury. *Am J Physiol* 274:F587-F595, 1998
55. Singh A, Kaushal V, Megyesi JK, et al: Cloning and expression of rat caspase-6 and its localization in renal ischemia/reperfusion injury. *Kidney Int* 62:106-115, 2002
56. Krajewska M, Wang HG, Krajewski S, et al: Immunohistochemical analysis of in vivo patterns of expression of CPP32 (caspase-3), a cell death protease. *Cancer Res* 57:1605-1613, 1997
57. Daemen M, Van't Veer C, Denecker G, et al: Inhibition of apoptosis induced by ischemia/reperfusion prevents inflammation. *J Clin Invest* 104:541-549, 2000
58. Kinoshita M, Tomimoto H, Kinoshita A, et al: Up-regulation of the Nedd2 gene encoding an ICE/Ced-3-like cysteine protease in the gerbil brain after transient global ischemia. *J Cereb Blood Flow Metab* 17:507-514, 1997
59. Asahi M, Hoshimaru M, Uemura Y, et al: Expression of interleukin-1 β converting enzyme gene family and bcl-2 gene family in the rat brain following permanent occlusion of the middle cerebral artery. *J Cereb Blood Flow Metab* 17:11-18, 1997
60. Bhat RV, DiRocco R, March VR, et al: Increased expression of IL-1 β converting enzyme in hippocampus after ischemia: Selective localization in microglia. *J Neurosci* 16:4146-4154, 1996
61. Cerretti DP, Kozlosky CJ, Mosley B, et al: Molecular cloning of the interleukin-1 beta converting enzyme. *Science* 25:97-100, 1992
62. Fantuzzi G, Dinarello CA: Interleukin-18 and interleukin-1 beta: Two cytokine substrates for ICE (caspase-1). *J Clin Immunol* 19:1-11, 1999
63. Melnikov VY, Eceder T, Fantuzzi G, et al: Impaired IL-18 processing protects caspase-1-deficient mice from ischemic acute renal failure. *J Clin Invest* 107:1145-1152, 2001
64. Daemen M, Denecker G, van't Veer C, et al: Activated caspase-1 is not a central mediator of inflammation in the course of ischemia-reperfusion. *Transplantation* 71:778-784, 2001
65. Melnikov VY, Faubel S, Siegmund B, et al: Neutrophil-independent mechanisms of caspase-1 and IL-18-mediated ischemic acute tubular necrosis in mice. *J Clin Invest* 110:1083-1091, 2002
66. Shi Y, Melnikov V, Schrier R, et al: Down-regulation of the calpain inhibitor protein, calpastatin, by caspases during renal ischemia. *Am J Physiol* 279:F509-F517, 2000
67. Nogue S, Miyazaki M, Kobayashi N, et al: Induction of apoptosis in ischemia-reperfusion model of mouse kidney: Possible involvement of Fas. *J Am Soc Nephrol* 9:620-631, 1998
68. Schelling JR, Cleveland RP: Involvement of Fas-dependent apoptosis in renal tubular epithelial cell deletion in chronic renal failure. *Kidney Int* 56:1313-1316, 1999
69. Donnahoo K, Meng X, Ayala A, et al: Early kidney TNF- α expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol* 277:R922-R929, 1999
70. Daemen M, Ven M, Heineman E, et al: Involvement of endogenous interleukin-10 and tumor necrosis factor- α in renal ischemia-reperfusion injury. *Transplantation* 67:792-799, 1999
71. Ramesh G, Reeves WB: TNF- α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. *J Clin Invest* 110:835-842, 2002
72. Knotek M, Rogacher B, Wang W, et al: Endotoxemic renal failure in mice: Role of tumor necrosis factor independent of inducible nitric oxide synthase. *Kidney Int* 59:2243-2249, 2001
73. Choi Y, Baranowska-Daca E, Nguyen V, et al: Mechanism of chronic obstructive uropathy: Increased expression of apoptosis-promoting molecules. *Kidney Int* 58:1481-1491, 2000
74. Gobe G, Zhang XJ, Willgross DA, et al: Relationship between expression of Bcl-2 gene and growth factors in ischemic acute renal failure in the rat. *J Am Soc Nephrol* 11:454-467, 2000
75. Basile DP, Liapis H, Hammerman MR: Expression of bcl-2 and bax in regenerating rat renal tubules following ischemic injury. *Am J Physiol* 272:F640-F647, 1997
76. Gross A, McDonnell JM, Korsmeyer SJ: Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev* 13:1899-1911, 1999
77. Li H, Zhu H, Xu CJ, et al: Cleavage of Bid by caspase-8 mediates the mitochondrial damage in the apoptosis. *Cell* 94:491-501, 1998
78. Luo X, Budihardjo I, Zou H, et al: Bid, a Bcl-2-interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94:481-490, 1998