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Mitochondrial Implication in Accidental and Programmed Cell Death: Apoptosis and Necrosis

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Both physiological cell death (apoptosis) and at least some cases of accidental cell death (necrosis) involve a two-step-process. At a first level, numerous physiological or pathological stimuli can trigger mitochondrial permeability transition which constitutes a rate-limiting event and initiates the common phase of the death process. Mitochondrial permeability transition (PT) involves the formation of proteaceous, regulated pores, probably by apposition of inner and outer mitochondrial membrane proteins which cooperate to form the mitochondrial PT pore complex. Inhibition of PT by pharmacological intervention on mitochondrial structures or mitochondrial expression of the apoptosis-inhibitory oncoprotein Bcl-2 thus can prevent cell death. At a second level, the consequences of mitochondrial dysfunction (collapse of the mitochondrial transmembrane potential, uncoupling of the respiratory chain, hyperproduction of superoxide anions, disruption of mitochondrial biogenesis, outflow of matrix calcium and glutathione, and release of soluble intermembrane proteins) can entail a bioenergetic catastrophe culminating in the disruption of plasma membrane integrity (necrosis) and/or the activation and action of apoptogenic proteases with secondary endonuclease activation and consequent oligonucleosomal DNA fragmentation (apoptosis). The acquisition of the biochemical and ultrastructural features of apoptosis critically relies on the liberation of apoptogenic proteases or protease activators from the mitochondrial intermembrane space. This scenario applies to very different models of cell death. The notion that mitochondrial events control cell death has major implications for the development of death-inhibitory drugs.

KEY WORDS: Mitochondrial transmembrane potential; permeability; transition; programmed cell death; proteases.

INTRODUCTION

Physiological cell death (PCD)[2] constitutes a strictly regulated ("programmed") device which is responsible for the removal of superfluous, aged, or damaged cells. In conditions of homeostasis, each mitosis is compensated for by one event of PCD. An abnormal resistance to PCD entails malformations, autoimmune disease, or cancer due to the persistence of superfluous, self-specific, or mutated cells, respectively. In contrast, enhanced removal of cells by PCD participates in acute diseases (intoxications, septic shock, anoxia), as well as in chronic pathologies (neurodegenerative and neuromuscular diseases, AIDS). PCD, whose morphological and biochemical phenotype is referred to as "apoptosis," is characterized by the action of catabolic enzymes, mostly hydrolases

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[2] Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocator; $\Delta\Psi_m$, mitochondrial inner transmembrane potential; mClCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PARP, poly (ADP-ribose) polymerase; PBR, peripheral benzodiazepine receptor; PCD, physiological cell death; PT, permeability transition; PS, phosphatidylserine; ROS, reactive oxygen species; VDAC, voltage-dependent anion channel; Z-VAD.fmk, *N*-benzyloxycarbonyl-Val-Ala-Asp.fluoromethylketone.

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(proteases and nucleases), within the limits of a near-to-intact plasma membrane. Thus, the cell actively contributes to its removal and undergoes a series of stereotyped biochemical and ultrastructural alterations (Table I). Apoptosis is the final outcome of multiple different death-inducing pathways. In mammalian cells, such apoptosis-triggering stimuli include interventions on second messenger systems, and ligation of certain receptors (Fas/APO-1/CD95, TGF-R, TNF-R, etc.) or, in the case of obligate growth factor receptors, the absence of receptor occupancy. In addition, suboptimal culture conditions (lack of essential compounds, shortage of nutrients, lack of oxygen), mild physical damage (radiotherapy), and numerous toxins (chemotherapy and toxins *stricto sensu*) can induce apoptosis (Barr and Tomei, 1994; Kroemer, 1995; Kroemer *et al.*, 1995; Thompson, 1995; Wertz and Hanley, 1996). Thus, apoptosis can result both from physiological and from pathological triggers. *In vivo*, cells undergoing apoptosis are recognized and removed by phagocytes before they undergo lysis. Phagocytic recognition of apoptotic cells (heterophagy) is facilitated by characteristic changes in plasma membrane structure, namely the loss of plasma membrane asymmetry with a consequent aberrant exposure of phosphatidylserine (PS) residues (normally only located in the inner membrane leaflet) on the cell surface.

In strict contrast to apoptosis, accidental cell death (necrosis) is always induced by nonphysiological effectors (toxins, physical or chemical damage) and thus constitutes a pathological phenomenon. Necrosis involves an early disruption of the plasma membrane before the cell can activate its proteases and nucleases. As a consequence, necrosis causes the liberation of cellular enzymes into the interstitium and thereby provokes inflammatory reactions *in situ* (Table I).

Although many authors have insisted on the paradigmatic opposition between apoptosis and necrosis, the difference between both modes of cell death remains ill defined, especially at the functional level (Table II). In particular, it appears that irreversible, severe changes in mitochondrial structure and function determine both the apoptotic and the necrotic modes of cell death. The present review will focus on recent evidence implicating mitochondria in physiological and accidental cell death.

MITOCHONDRIA IN CELL DEATH: PHENOMENOLOGICAL OBSERVATIONS

Massive changes in mitochondrial ultrastructure are pathognonomic for necrosis. Such alterations include swelling of the matrix, dilatation of cristae, and disruption of membranes. In contrast, electron microscopic alterations are far more discrete in mitochondria from cells undergoing apoptosis. However, mild swelling of the matrix coupled to a reduction in electron density has been observed in several models of apoptosis. More importantly, in many cases of necrosis and in more than fifty different models of apoptosis, a characteristic disruption of the mitochondrial transmembrane potential ($\Delta\Psi_m$) has been

Table I. Apoptosis Versus Necrosis: a Comparison

Apoptosis	Primary necrosis
Physiological or pathological (subnecrotic damage)	Accidental
Susceptibility tightly regulated	Always pathological
Plasma membranes intact until late stage	Unregulated or poorly regulated
Heterophagic elimination	Membranes destroyed early
No inflammation	Leakage of cell contact
Cellular enzymes participate causing characteristic biochemical or morphological features including:	Inflammation
	No stereotypical biochemical or morphological features
• chromatin condensation (pyknosis)	
• nuclear fragmentation (karyorrhexis)	
• regular DNA fragmentation pattern (endonucleolysis)	
	Secondary necrosis
• selective protein degradation (proteolysis)	
• subtle changes in plasma membranes	Secondary to apoptosis when dying cells
• cell shrinkage	fail to be removed by heterophagy

Table II. Arguments Against the Antithesis Between Apoptosis and Necrosis

Argument	Reference
After apoptosis, cells undergo secondary necrosis.	(Kroemer *et al.*, 1995; Thompson, 1995)
The same toxin can induce apoptosis at a low dose and primary necrosis at a high dose.	(Kroemer, 1995; Kroemer *et al.*, 1995)
Many pathologies thought to be mediated by necrosis also involve apoptosis (apoplexy, myocardial infarction, ischemia-reperfusion damage, etc.).	(Martinou *et al.*, 1994; Itoh *et al.*, 1995; Fliss and Gattinger 1996; Simonian *et al.*, 1996)
The oncoprotein Bcl-2 can inhibit both apoptosis and primary necrosis.	(Kane *et al.*, 1993; Martinou *et al.*, 1994; Shimizu *et al.*, 1995; Shimizu *et al.*, 1996)
Overexpression of Bax causes apoptosis and, in the absence of protease activation, necrosis.	(Xiang *et al.*, 1996)
Mitochondrial permeability transition is involved in both apoptosis and necrosis.	(Kroemer *et al.*, 1995; Kroemer, 1997; Kroemer *et al.*, 1997)

observed (reviewed by Kroemer *et al.*, 1995; Petit *et al.*, 1995; Zamzami *et al.*, 1995a; Castedo *et al.*, 1996; Macho *et al.*, 1996; Kroemer 1997). In the case of apoptosis, this $\Delta\Psi_m$ collapse precedes the activation of endonucleases as well as the exposure of PS at the cell surface [Kroemer *et al.*, 1997; Susin *et al.*, 1997). All presently available data are compatible with the notion that $\Delta\Psi_m$ dissipation constitutes an early, obligatory, and irreversible step of the apoptotic process. In other terms, the $\Delta\Psi_m$ disruption marks a point of convergence of distinct apoptosis induction pathways and constitutes the point-of-no-return of apoptosis. In accord with the fact that maintenance of an intact $\Delta\Psi_m$ is necessary for mitochondrial biogenesis, cells manifest an arrest in mitochondrial mRNA and protein synthesis early during the apoptotic process (Osborne *et al.*, 1994; Vayssière *et al.*, 1994). After $\Delta\Psi_m$ disruption, when cells become surface PS^+ and start to digest their DNA, mitochondria hyperproduce reactive oxygen species (ROS) on the uncoupled respiratory chains and locally oxidize cardiolipin molecules from the inner mitochondrial membrane (Zamzami *et al.*, 1995a, b; Castedo *et al.*, 1996). It appears that the pre-apoptotic $\Delta\Psi_m$ collapse is associated with a loss of mitochondrial membrane integrity, as suggested by the cytosolic efflux of cytochrome *c*, a protein that under normal circumstances is confined to the intermembrane space (Liu *et al.*, 1996). In conclusion, mitochondria undergo profound structural and functional changes early during the death process. Such changes are more explicit, at the structural level, during necrosis, yet also concern, at the functional and biochemical levels, an early stage of apoptosis.

MITOCHONDRIA IN CELL DEATH: PHARMACOLOGICAL EVIDENCE

Numerous observations indicate that induction of a $\Delta\Psi_m$ collapse triggers cell death, whereas stabilization of the $\Delta\Psi_m$ prevents cell death. Currently available data fit well with the idea that permeability transition (PT) accounts for the death-associated $\Delta\Psi_m$ dissipation. PT is now thought to be due to the formation of dynamic multiprotein ensembles (the so-called "PT pores" or "mitochondrial megachannels") at inner/outer membrane contact sites. These complexes are currently believed to involve cytosolic proteins (hexokinase), outer membrane proteins (peripheral benzodiazepine receptor [PBR]; porin, also called voltage-dependent anion channel [VDAC]), intermembrane proteins (creatine kinase), at least one inner membrane protein (the adenine nucleotide translocator, ANT), and at least one matrix protein (cyclophilin D) (McEnery *et al.*, 1992; Zoratti and Szabò, 1995; Bernardi and Petronilli, 1996; Beutner *et al.*, 1996; Brustovetsky and Klingenberg, 1996) (Fig. 2). Depending on different physiological effectors (Table III), the PT pore complex can adopt an open or closed conformation. Substances which specifically act on mitochondrial structures to induce PT can trigger cell death. Thus, protoporphyrin IX (PPIX), a ligand of the peripheral benzodiazepine receptor (PBR), one of the constituents of the PT pore complex, induces apoptosis in lymphoid cells (Marchetti *et al.*, 1996a, c) and necrosis in hepatocytes (Pastorino *et al.*, 1994). Similarly, the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (*m*CICCP), which incorporates into the inner mitochondrial membrane to disrupt the $\Delta\Psi_m$, causes apoptosis in lymphoid cells (Susin *et al.*, 1996). Pharma-

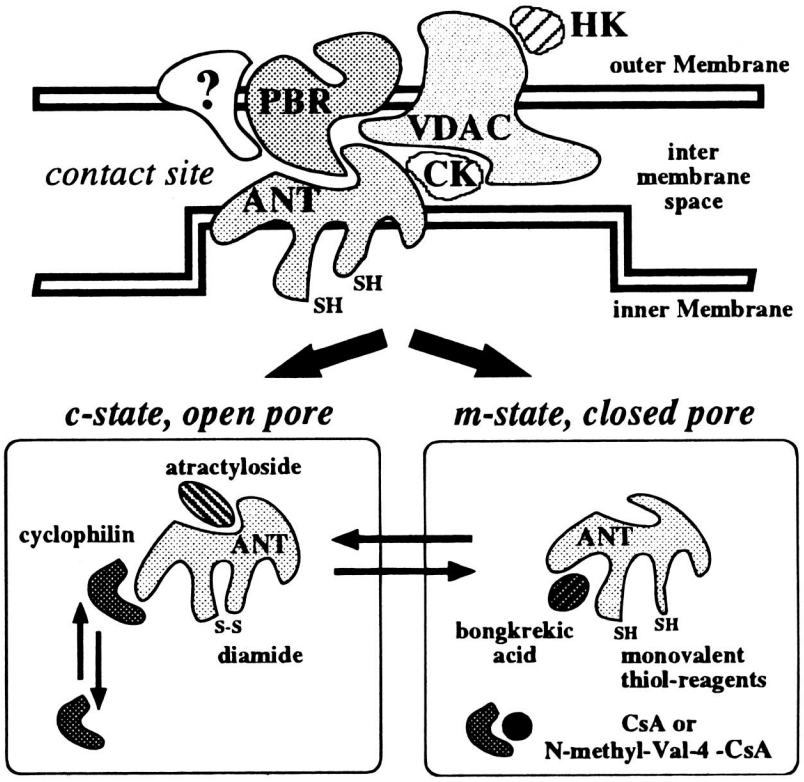

Fig. 1. Hypothetical model of the PT pore complex. The PT pore complex is made up by proteins from the cytosol (e.g., hexokinase, HK, which facilitates PT), the outer mitochondrial membrane (peripheral benzodiazepine receptor, PBR; voltage-dependent anion channel, VDAC), the intermembrane space (creatine kinase, CK, which inhibits PT), the inner membrane (adenine nucleotide translocator, ANT), and the matrix (cyclophilin D). The exact stoichiometry and the sites of interaction of these proteins are not known. Moreover, additional, yet unknown (?) proteins could participate in the regulation of the complex. The conformation of the ANT, which is influenced by its endogenous (ADP, ATP) and exogenous ligands (atractyloside, bongkrekic acid), co-determines the probability of PT pore opening. The interaction of cyclophilin D with inner membrane proteins is also critical for PT pore opening. This interaction is inhibited by cyclosporin A (CsA) and the nonimmunosuppressive CsA derivative N-methyl-Val-4-CsA. At present, it is not clear whether cyclophilin D interacts with the ANT or other inner membrane structures. The opening of the PT pore is also facilitated by the oxidation of vicinal thiols located at the matrix site of an inner mitochondrial membrane protein (possibly the ANT). This oxidation is prevented by monovalent thiol-reactive agents such as monochlorobiman or chloromethyl-X-rosamine. It remains elusive whether these critical thiols are located within the ANT or other, yet unknown, PT pore components.

cological inhibitors of PT can prevent cell death in numerous different models (Table IV). Thus, drugs designed to prevent PT via specific interaction with the adenine nucleotide translocator (bongkrekic acid), matrix cyclophilin D (cyclosporin A and *N*-methyl-4-Val-cyclosporin A), or matrix thiols (chloromethyl-X-rosamine) can inhibit cell death (apoptosis or necrosis) in different cell types (Table IV). In addition to preventing the death-associated $\Delta\Psi_m$ disruption, these drugs prevent all postmitochondrial manifestations of apoptotic cell death, at the levels of redox balance, PS exposure, and activation of proteases and nucleases (Castedo *et al.*, 1996; Marchetti *et al.*, 1996a, b; and unpublished data). These pharmacological studies indicate that PT-mediated $\Delta\Psi_m$ disruption constitutes a critical coordinating event of the death process.

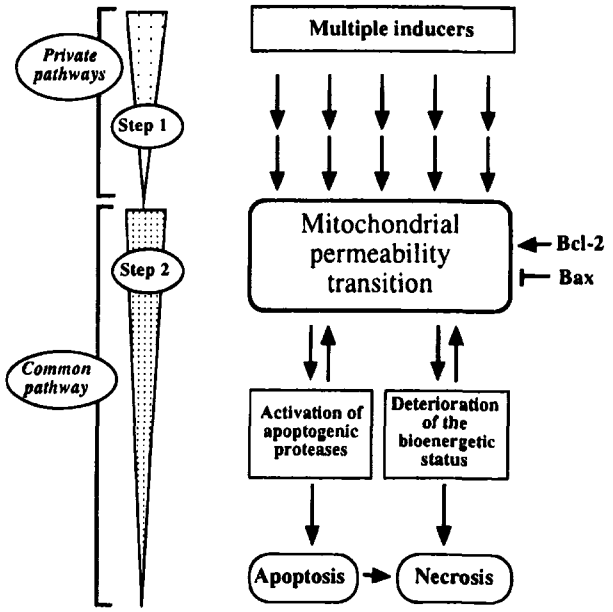

Fig. 2. A hypothetical two-step model of cell death. At the first step multiple different signal transduction pathways or metabolic conditions cause mitochondrial permeability transition (PT). These pathways are "private" in the sense that they are stimulus-dependent. PT is inhibited by Bcl-2 and facilitated by Bax. PT initiates the common pathway of the death process and causes both the activation of apoptogenic proteases and the deterioration of the bioenergetic status. Depending on the rapidity of the latter, as well as an the availability of apoptogenic proteases, either primary necrosis or apoptosis results from PT. Note that apoptosis is finally followed by secondary necrosis. Several of the consequences of PT themselves favor PT. Thus, once PT has been triggered above a threshold level, it self-amplifies via one or several positive feedback loops (bidirectional arrows). This explains why PT tends to react in an all-or-nothing fashion.

MITOCHONDRIA IN APOPTOSIS: DATA FROM CELL-FREE SYSTEMS

Cell-free systems, that is, *in vitro* cultures of disassembled cellular components, can mimic some features of apoptosis. It has been shown that cytosols from cells undergoing apoptosis contain apoptogenic proteins capable of provoking isolated nuclei to undergo chromatin condensation and DNA fragmentation (Lazebnik *et al.*, 1995). A similar apoptogenic activity is encountered in whole cytoplasmic preparations of normal cells, and this activity is associated with mitochondria (Newmeyer *et al.*, 1994; Martin *et al.*, 1995). Cytochrome *c*, a mitochondrial intermembrane protein, has been found to exert a co-apoptogenic activity (Liu *et al.*, 1996). Thus, cytochrome *c* itself is insufficient to cause nuclear apoptosis *in vitro*. However, it acts in conjunction with yet to be characterized cytosolic factors to activate apoptogenic proteases *in vitro* (Liu *et al.*, 1996). Our group has employed a different approach to investigate the possible link between mitochondrial PT and nuclear apoptosis. When mixing together purified mitochondria and nuclei, in the absence of additional cytosolic compounds, nuclei only manifest apoptotic changes when mitochondria are induced to undergo PT (Marchetti *et al.*, 1996d; Zamzami *et al.*, 1996). Normal mitochondria are inert in this system (Marchetti *et al.*, 1996d; Zamzami *et al.*, 1996). We have then shown that mitochondria undergoing PT release a pre-formed soluble *a*poptosis-*i*nducing *f*actor (AIF). In contrast to cytochrome *c*, AIF suffices to cause nuclear apoptosis *in*

Table III. Inducers and Inhibitors of Apoptosis Acting on Permeability Transition Pores

Inducers of permeability transition		Inhibitors of permeability transition	
Substance	Target	Substance	Target
Protoporphyrin IX	PBR	Cyclosporin A	Cyclophilin D
Atractyloside	ANT	Bongkrekic acid	ANT
Calcium	Unknown	Calcium chelators	Calcium
Oxidizing agents	Matrix thiols?	Monochlorobiman Monobromobiman	Matrix thiols?
Protonophores	Inner mitochondrial membrane	Chloromethyl-X-rosamin	Matrix thiols
Disulfide bridge-forming agents	Matrix thiols	Calpain inhibitors	Mitochondrial calpain-like protease
Proteases	Unknown		
Protons	Reversible histidyl protonation	Phosphotyrosine	Tyrosine kinases?

Abbreviations: ANT, adenine nucleotide translocator; PBR, peripheral benzodiazepine receptor.

Table IV. Pharmacological Evidence for the Involvement of Permeability Transition in Cell Death

Cell type	Inducer of cell death (underlying principle)	Inhibitor of $\Delta\Psi_m$ disruption and apoptosis	Reference
Myocardial	Ischemia reperfusion damage causes necrosis and apoptosis	Cyclosporin A	(Nazareth *et al.*, 1991; Griffiths and Halestrup 1993)
	Tetramethylrhodamine ethyl ester (intramitochondrial formation of ROS)	Cyclosporin A	(Leyssens *et al.*, 1996)
Hepatocytes	Protoporphyrin IX (ligand of the PBR) causes necrosis	Calcium chelators	(Pastorino *et al.*, 1994)
	Rotenone, cyanide (chemical anoxia) cause necrosis	Cyclosporin A, L-carnitine	(Pastorino *et al.*, 1993)
	Cyanide (chemical anoxia)	Cyclosporin A + calcium chelation	(Pastorino *et al.*, 1995)
	t-butylhydroperoxide (a pro-oxidant) induces necrosis	Cyclosporin A	(Kass *et al.*, 1992)
		Trifluoperazine	(Nieminen *et al.*, 1995)
		Cyclosporin A + trifluoroperazine	(Imberti *et al.*, 1993)
	Menadion (oxidative stress, necrosis)	Cyclosporin A, ruthenium red	(Saxena *et al.*, 1995)
	Paraquat (oxidative stress, necrosis)	Cyclosporin A	(Constantini *et al.*, 1995)
	Glycochenodeoxycholate	Ursodeoxycholate	(Botla *et al.*, 1995)
		Cyclosporin A + trifluorperazine	
	1-methyl-4-phenylpyridinium	Cyclosporin A	(Snyder *et al.*, 1992)
Neurons	Glutamate (excitotoxin) causes apoptosis and necrosis	Cyclosporin A	(Ankarcrona *et al.*, 1995; Schinder *et al.*, 1996)
	1-methyl-4-phenylpyridinium (excitotoxin)	Cyclosporin A	(Packer *et al.*, 1996)
	Transient forebrain ischemia causes apoptosis and necrosis	Cyclosporin A	(Shiga *et al.*, 1992; Uchino *et al.*, 1995)
Proximal tubular cells	1,2-dichlorovinyl-L-cysteine (oxidative stress)	Calcium chelators, ruthenium red, antioxidants	(van de Water *et al.*, 1994)
L929 fibrosarcoma	Tumor necrosis factor causes necrosis	Cyclosporin A plus trifluoperazine	(Pastorino *et al.*, 1996)
Thymocytes (apoptosis)	Protoporphyrin IX (PBR ligand)	Bongkrekic acid (ANT ligand)	(Marchetti *et al.*, 1996a, c)
	Dexamethasone (glucocorticoid)	Bongkrekic acid, CMXRos	(Marchetti *et al.*, 1996a, bb)
	Etoposide (topoisomerase inhibitor)	Bongkrekic acid, CMXRos	(Marchetti *et al.*, 1996a, b)
	γ-irradiation (DNA damage)	Bongkrekic acid, CMXRos	(Marchetti *et al.*, 1996a, b)
	Diamide (thiol crosslinking)	CMXRos	(Marchetti *et al.*, 1996a, b)
	Low dose thapsigargin (increase in cytosolic calcium)	Cyclosporin A	(Waring and Beaver, 1996)
	Nitric oxide (NO)	Bongkrekic acid	S. Hortelano and G. Kroemer, unpublished
Drosophila ommatidia	Normal development causes apoptosis of excess cells in the eye	Bongkrekic acid	A. Goldsborough and G. Kroemer, unpublished

vitro. AIF appears to be a protease which is neutralized by the protease inhibitor *N*-benzyloxycarbonyl-Val-Ala-Asp.fluoromethylketone (Z-VAD.fmk), a substance which also inhibits nuclear apoptosis in cells (Susin *et al.*, 1996), underlining the probable *in vivo* relevance of AIF. Mitochondria induced to undergo PT also release other putative apoptogenic factors including cytochrome *c* (unpublished results). Both AIF and cytochrome *c* are confined to the intermembrane space of mitochondria. However, AIF is not identical with cytochrome *c*, and the relationship between both factors remains elusive. Irrespective of the putative relative impact of AIF and cytochrome *c* on apoptosis occurring in cells, the data obtained in cell-free systems underline the functional impact of apoptogenic factors that under normal circumstances are confined to mitochondria.

MITOCHONDRIA AS TARGETS OF DEATH-REGULATORY PROTEINS: Bcl-2 AND BAX

Bcl-2 belongs to a growing family of apoptosis-regulatory proteins. The *bcl-2* gene family encodes

both apoptosis-inhibitory proteins (prototype: Bcl-2) and apoptosis-inducing proteins (prototype: Bax). These proteins form homo- or heterodimers, and dimerization via specific protein–protein interactions is important for their function (Cory, 1995; Reed, 1995; Yang and Korsmeyer, 1996). A disequilibrium in the expression of these gene products is involved in the development of about half of human cancers. Bcl-2 possesses a C-terminal hydrophobic region that allows for its incorporation into the nuclear envelope, the endoplasmic reticulum, and the mitochondrial outer membrane. Apparently, this latter localization is functionally important. Removal of the membrane anchoring domain tends to reduce the efficacy of Bcl-2 and Bax as death regulators (Hockenbery *et al.*, 1993; Zha *et al.*, 1996; Kroemer *et al.*, 1997). Replacement by membrane stretches causing specific targeting of Bcl-2 or Bax into the outer mitochondrial membrane (but not other intracellular membranes) restores their apoptosis-regulatory potential (Nguyen *et al.*, 1994; Greenhalf *et al.*, 1996; Zha *et al.*, 1996; Zhu *et al.*, 1996). Additional evidence for mitochondrial effects of Bcl-2 has been obtained by studying mitochondrial function in cells hyperexpressing Bcl-2. Bcl-2 prevents the pre-apoptotic $\Delta\Psi_m$ collapse in a variety of different cellular models, including cells lacking a nucleus (so-called cytoplasts) (Zamzami *et al.*, 1995a; Castedo *et al.*, 1996; Shimizu *et al.*, 1996; Zamzami *et al.*, 1996; Decaudin *et al.*, 1997). This $\Delta\Psi_m$-stabilizing effect is shared by an apoptosis-inhibitory Bcl-2 analog, Bcl-X_L (Decaudin *et al.*, 1997). In contrast, overexpression of Bax induces $\Delta\Psi_m$ disruption (Xiang *et al.*, 1996). It has also been shown that Bcl-2 modulates the calcium influx into and efflux from the mitochondrial matrix (Baffy *et al.*, 1993; Murphy *et al.*, 1996). Evidence from cell-free systems emphasizes the mitochondrial effects of Bcl-2. Thus, incorporation of recombinant Bcl-2 into mitochondria reduces their apoptogenic capacity (Newmeyer *et al.*, 1994). Mitochondria isolated from cells which hyperexpress Bcl-2 are protected against the PT-inducing effect of atractyloside, protoporphyrin IX, the pro-oxidant *tert*-butylhydroperoxide, and the protonophore *m*CICCP (Marchetti *et al.*, 1996c; Susin *et al.*, 1996; Zamzami *et al.*, 1996). When inhibiting PT, Bcl-2 also prevents the PT-associated release of AIF (Susin *et al.*, 1996; Zamzami *et al.*, 1996). Mitochondria from *bcl-2*-transfected cells contain similar amounts of AIF as mitochondria from vector-only-transfected control cells (Susin *et al.*, 1996). Thus, Bcl-2 does not affect the formation of AIF yet does inhibit its release from mitochondria. We and others have failed to observe any major effects of Bcl-2 on the action of apoptogenic factors including AIF (Newmeyer *et al.*, 1994; Susin *et al.*, 1996). Bcl-2 present in the nuclear envelope does not inhibit the apoptogenic effect of AIF (Susin *et al.*, 1996).

In conclusion, molecular genetic, functional, and biochemical evidence indicates that proteins from the Bcl-2 family exert their apoptosis-regulatory function via an effect on mitochondria, presumably via inhibiting PT. The fact that Bcl-2 inhibits a mitochondrial phenomenon implicated in both apoptosis and necrosis may explain why Bcl-2 can negatively affect both modes of cell death. At present, it remains unknown how Bcl-2 controls PT. Speculatively, it could do so by controlling ion fluxes (Baffy *et al.*, 1993; Muchmore *et al.*, 1996; Murphy *et al.*, 1996), protein kinases (Wang *et al.*, 1996), or interaction with proteins from the PT pore complex. This latter possibility is emphasized by the fact that Bcl-2 locates to the inner/outer membrane contact site, where PT pores are expected to form (Krajewski *et al.*, 1993). Moreover, in lymphoid cell lines, the expression levels of Bcl-2 and PBR, one of the putative PT pore constituents, correlate in a quasi-stoichiometric fashion (Carayon *et al.*, 1996), suggesting that Bcl-2 could interact with the PBR or a PBR-associated protein.

CONCLUSIONS AND PERSPECTIVES

As discussed in this review, mitochondrial PT constitutes a critical coordinating event of both apoptosis and necrosis. In a two-step model of cell death, this PT would occupy the first position. At a second level, the downstream consequences of PT would determine the mode of cell death. PT has several metabolic consequences that are self-sufficient to cause cell death: uncoupling of oxidative phosphorylation and a major disturbance of redox regulation with an increase of superoxide anion generation (Vayssière *et al.*, 1994; Zamzami *et al.*, 1995a). In addition, PT is accompanied by the mitochondrial release of intermembrane proteins that may be toxic for cells (Susin *et al.*, 1996; Zamzami *et al.*, 1996). Thus, cytochrome *c* released from mitochondria undergoing PT may cooperate with yet unknown factors to activate the protease CPP32/Yama/Apopain and to induce nuclear apoptosis (Liu *et al.*, 1996). Mitochondria also contain an apoptogenic protease ("apoptosis-inducing factor," AIF) that suffices to induce apoptotic changes in isolated nuclei *in vitro*, in the absence of additional cyto-

plasmic components (Susin *et al.*, 1996; Zamzami *et al.*, 1996). Our unpublished data suggest that cells that have been driven to undergo apoptosis may die from necrosis when apoptogenic proteases fail to come into action. Thus, when PT is induced in a massive, rapid fashion heavily compromising ATP supply, necrosis (that is, primary disruption of the plasma membrane) occurs before apoptogenic proteases are activated and can act on nuclear and cytoplasmic substrates. In contrast, induction of PT in a more smooth, protracted fashion allows for the activation and action of specific proteases (AIF, CPP32, and other downstream proteases) before ATP depletion and other consequences of PT such as enhanced superoxide anion generation cause cell death. In other terms, the intensity of the PT-inducing stimulus may determine which among two major consequences of PT wins the race: a bioenergetic catastrophe culminating in necrosis or the activation/action of apoptogenic proteases. This scenario would be compatible with the finding that many drugs induce necrosis at high doses and apoptosis at lower ("subnecrotic") doses (Kroemer, 1995; Kroemer *et al.*, 1995). This view of cell death would also be compatible with the fact that Bcl-2 can prevent both necrosis and apoptosis (Kane *et al.*, 1993; Martinou *et al.*, 1994; Shimizu *et al.*, 1996) and that overexpression of Bax can induce apoptosis and, in the absence of protease activation, necrosis (Xiang *et al.*, 1996).

The above two-step model of cell death may have a major impact on the development of drugs designed for the prevention of cell death. Sudden, massive cell death (apoptosis and necrosis) is involved in a number of acute pathologies (anoxia, ischemia/reperfusion damage, intoxication, septic shock) whereas elevated apoptotic cell loss is observed in a variety of chronic degenerative diseases (neurodegenerative or neuromuscular diseases, AIDS). In contrast, hyperexpression of apoptosis-inhibitory Bcl-2 homologues (or low expression of their apoptosis-inducing counterparts) can contribute to oncogenesis and the development of chemotherapy-resistant tumor cells. In all these disorders, mitochondrial PT pores constitute putative targets for future pharmacological interventions. It is thus conceivable that the exploration of PT pore structure and function can lay the grounds for the therapeutic correction of disease-mediating dysregulations in cell death control.

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