

Review

Necrosis: a specific form of programmed cell death?

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Received 10 July 2002, revised version received 11 September 2002

Abstract

For a long time necrosis was considered as an alternative to programmed cell death, apoptosis. Indeed, necrosis has distinct morphological features and it is accompanied by rapid permeabilization of plasma membrane. However, recent data indicate that, in contrast to necrosis caused by very extreme conditions, there are many examples when this form of cell death may be a normal physiological and regulated (programmed) event. Various stimuli (e.g., cytokines, ischemia, heat, irradiation, pathogens) can cause both apoptosis and necrosis in the same cell population. Furthermore, signaling pathways, such as death receptors, kinase cascades, and mitochondria, participate in both processes, and by modulating these pathways, it is possible to switch between apoptosis and necrosis. Moreover, antiapoptotic mechanisms (e.g., Bcl-2/Bcl-x proteins, heat shock proteins) are equally effective in protection against apoptosis and necrosis. Therefore, necrosis, along with apoptosis, appears to be a specific form of execution phase of programmed cell death, and there are several examples of necrosis during embryogenesis, a normal tissue renewal, and immune response. However, the consequences of necrotic and apoptotic cell death for a whole organism are quite different. In the case of necrosis, cytosolic constituents that spill into extracellular space through damaged plasma membrane may provoke inflammatory response; during apoptosis these products are safely isolated by membranes and then are consumed by macrophages. The inflammatory response caused by necrosis, however, may have obvious adaptive significance (i.e., emergence of a strong immune response) under some pathological conditions (such as cancer and infection). On the other hand, disturbance of a fine balance between necrosis and apoptosis may be a key element in development of some diseases.

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Keywords: Apoptosis; Necrosis; Programmed cell death; Signal transduction

Introduction

In the early 1970s the discovery of new patterns of cell death led to emergence of the concept of apoptosis [1]. During apoptosis there were remarkably arranged morphological and biochemical events while necrosis was apparently deranged (or accidental) form of cell death [2]. Apoptosis was later considered as an example of a programmed cell death (PCD). The conception claims that cell death from pathophysiological stimuli is a particular case of the evolutionary conservative mechanism of cell elimination upon morphogenetic and homeostatic signals in animals and plants. A sensational success of the conception was also incited by a discovery (in nematode *Caenorhabditis el-*

egans) of genes responsible for biochemical mechanisms of suicidal cell destruction [3].

The PCD and apoptosis were initially considered to be the processes that are strictly dependent on expression of new (“death”) genes [2]. This led to almost complete disregard of epigenetic mechanisms of cell death, that is, mechanisms that do not require de novo protein synthesis. One such mechanism (Apo-1/Fas-induced death of lymphoid cells) was described in late 1980s [4,5]. The most striking finding was that characteristic apoptotic changes could be observed even in anucleated cells (cytoplasts); that is, they were independent on the nucleus [6,7]. These data have blurred a sharp contrast between apoptosis and necrosis, because the latter process was apparently independent of expression of new genes.

Morphologically, necrosis is quite different from “classical” apoptosis. During necrosis cells first swell, and then

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the plasma membrane collapses and cells are rapidly lysed. During apoptosis cells first shrink and their nuclei condense, and then they disintegrate into well-enclosed apoptotic bodies. Cell swelling during necrosis is emphasized in the term “oncosis” (derived from “oncos,” meaning swelling), the term for cell death opposite to apoptosis [8]. Biochemical hallmarks of apoptosis such as activation of specific proteases (caspases) and oligonucleosomal DNA fragmentation are usually absent in necrotic cells. However, improvement of methods of differentiation of apoptosis and necrosis revealed that there are many examples when some biochemical and morphological characteristics of both modes of cell death can be found in the same cell. This indicates that there is a spectrum of suicidal programs in cells, and “classical” necrosis and apoptosis are the extremes of the spectrum. In this review the term “apoptosis” will be attributed to processes of cell elimination without apparent disruption of the plasma membrane, while necrosis is cell death accompanied by a rapid efflux of cell constituents in extracellular space. Of note, however, is that in vitro apoptosis finally leads to plasma membrane permeabilization as well (“secondary necrosis”), but it does not occur in vivo since apoptotic cells are digested by macrophages or surrounding cells before their plasma membrane becomes disrupted. We will discuss mechanisms of necrosis, the consequences of this form of cell death for an organisms, and the possibility of modulating this process.

Physiological and pathophysiological stimuli leading to necrosis

Under extreme conditions tissues and cells die through unregulated processes of destruction of membranes and cytosol. This observation led to the assumption that when cell destruction is accompanied by rapid disruption of the plasma membrane, cytoplasmic structures, and the nucleus, it indicates that the death is passive and unregulated. However, such conclusion disregards many phenomena where necrotic cell death is a regulated process activated by specific physiological and pathological conditions.

Among the agents that can induce necrosis are various viruses, bacteria, and protozoa (Table 1). Necrosis can be activated by bacterial toxins [9,10] and components of immune defense, such as complement [11], activated natural killers [12], and peritoneal macrophages [13]. The pathogen-induced necrotic programs in cells of immunological barriers (e.g., intestine mucosa) may alleviate invasion of pathogens through the surfaces affected by inflammation [14] and, in the case of intracellular pathogens, to avoid “altruistic” apoptotic suicide that can prevent pathogen propagation [15] (see “Necrotic death is a regulated cellular response to stress and its physiological consequences” section for discussion).

Death of neurons that accompanies some brain diseases (Table 1) is an example of execution of a wide variety of

Table 1
Pathologies associated with necrotic cell death

Pathology	Cells/stimuli	Ref.
Infection	Lymphocytes/HIV virus	[194; 195]
	Hepatocytes/ <i>Mycobacterium avium</i>	[14]
	Neutrophils/ <i>Shigella flexneri</i>	[196]
	Macrophages/ <i>Salmonella typhi</i>	[197]
Alzheimer disease Creutzfeldt–Jakob disease	Neurons	[198]
	Neurons	[199]
Epilepsy	Neurons	[200]
Inflammatory diseases	Islet cells/diabetes	[22]
	Neutrophils, endothelial cells/ inflammatory cytokines	[201]
	Hepatocytes	[202]
Ischemia	Various cells	

lethal programs often caused by release of excitotoxins (see “Extracellular mediators, ligands, and receptors” section). Pathological conditions that are characterized by inadequate secretion of cytokines, nitric oxide (NO), and reactive oxygen species (ROS) are also accompanied by intense necrotic death of cells (Table 1). A classic example of necrotic conditions is ischemia that leads to a drastic depletion of oxygen, glucose, and other trophic factors and evokes massive necrotic death of endothelial cells and non-proliferating cells of surrounding tissues (neurons, cardiomyocytes, renal cells, etc.).

Recent cytological data indicate that necrotic death occurs not only during pathological events, but it is also a component of some physiological processes. For example, during renewal of the small intestine, both apoptosis and necrosis of enterocytes contribute to cell loss [16]. Similarly, in the large intestine, lower regions of crypts commonly contain isolated necrotic colonocytes, which also indicates that necrosis contributes to normal cell loss [17]. Follicular maturation during oogenesis involves, along with apoptosis, necrotic cell death [18]. Activation-induced death of primary T lymphocytes, an important constituent of negative selection in immune response, is caspase-independent and necrotic by morphology [19]. This may explain why transgenic mice expressing viral inhibitors of caspases (and, therefore, apoptosis) do not develop hyperplasia and autoimmune disease [20]: apparently, autoreactive T cells die via a necrotic pathway.

Furthermore, genetic experiments also indicate that necrotic cell death can potentially substitute apoptosis during normal development. For instance, genetic deletion of two key caspases, caspase-3 and caspase-9, did not affect normal loss of spinal cord and brain stem neurons during development, although it caused marked perturbation in morphology of the developing forebrain [20]. The most striking example is loss of interdigital cells in the mouse embryo, a paradigm of programmed cell death. When apoptosis was inhibited genetically, or by drugs, interdigital cell

death, although delayed, can still proceed [21]. Moreover, in normal mice some interdigital cells were found to die with a necrotic morphology [21].

Therefore, these reports undoubtedly demonstrate the occurrence of necrotic cell death not only during many pathological processes, but also during normal processes such as tissue renewal, embryogenesis, and immune response.

Receptors, messengers, and executors of necrotic program

Extracellular mediators, ligands, and receptors.

Among the agents that are capable to initiate necrosis-like programs are cytokines secreted by affected tissues during inflammation and infection. In a cellular model of diabetes, pancreatic β -cells of isolated Lanhengans islets die through both apoptosis and necrosis when exposed to a combination of the cytokines IL-1 β , TNF- α , and IFN- γ [22]. Cultured human chondrocytes undergo necrotic death after exposure to these cytokines and lipopolysaccharide, but antioxidants switched the lethal program to apoptosis [23] (see also “Redox signaling pathways” section).

Members of the TNF receptor family (TNF, FAS, TRAIL) may initiate not only apoptotic, but also necrotic cell death. Ligation of FAS caused caspase-independent cell death in activated T lymphocytes, a process apparently involved in immune response [19]. TRAIL ligand of death receptors DR4 and DR5 can also cause necrosis [19]. The fibrosarcoma cell line L929 is especially sensitive to the necrotic effect of TNF. This necrosis was dependent on death domain of TNFR-55 [24], and inhibition of caspases in these cells did not prevent TNF-induced death [25]. When L929 cells were transfected with the FAS gene, they began to die via apoptosis after anti-FAS addition, but maintained sensitivity to the necrotic effect of TNF [26]. This result indicates that apoptotic and necrotic programs can coexist in the same cell.

Activation of purinergic receptors such as P2Z by exogenous ATP may be another stimulus for necrotic cell death. In mesangial cells ATP induced formation of pores in plasma membrane, which led to necrosis and apoptosis [27]. Similarly, *Pseudomonas aeruginosa* caused necrotic death of macrophages in culture through activation of purinoreceptors [28].

Excitation of different subtypes of glutamate receptors plays an important role in choice of form of cell death [29]. Model experiments with excitotoxins (AMPA, NMDA, kainate) demonstrated that, depending on certain cell types, they die either via apoptosis or necrosis [30]. The intensity of necrotic destruction can be regulated by antagonists of glutamate or nonglutamate receptors [31,32] as well as by other downstream events (see next section).

Under some specific conditions survival factors (insulin or NGF) can initiate the necrotic program in neuronal cells

[33,34]. Such a paradoxical effect of NGF may be due to its binding to p75 (NTR), a member of TNF receptor superfamily, which is possible in the absence of or upon inhibition of TrkA receptors [35]. Activation of glucocorticoid receptors can be either antinecrotic (e.g., in case of macrophage-induced necrosis of target cells [13]) or pronecrotic (e.g., in necrosis of serum-deprived glioma cells [36]).

Thus, various intercellular mediators and their receptors, along with other responses, can activate both apoptosis and necrosis. Execution of these programs is dependent on type and intensity of stimulus as well as biochemical phenotype of a cell. There are also specific receptors and mechanisms not only for execution of cell death, but also for autocrine and paracrine protection of cells from conversion of a stressful signal to suicidal.

Ion channels and lipids

Calcium ions are the most powerful inductors and mediators of cell death. Removal of Ca²⁺ from medium (by chelators EGTA or BAPTA) protects cells from necrosis induced by starvation and anoxia [37]. It does not indicate just passive diffusion of the ions through membrane since both influx of extracellular Ca²⁺ and efflux of Ca²⁺ from intracellular depots depend on functioning of the specific channels. The blockade of these channels by antagonists (benidipin, etc.) significantly reduced cell death under starvation [34,37] or rotavirus [38].

Lipids and some products of their peroxidation can also induce necrotic cell death. For example, oxidized low-density lipoproteins (ox-LDL) induced necrosis, which was inhibited by Ca²⁺ chelators [39,40]. Oxidized sterols induced necrosis in fibroblasts but apoptosis in endothelial and smooth muscle cells [41]. An active product of lipid peroxidation, 4-hydroxynenal, induced necrotic death in neuronal cell culture, which could be inhibited by BAPTA and ruthenium red [42]. Accumulation of ceramide, a product of sphingomyelin hydrolysis, is early cellular response to variety of stresses. Ceramide and its penetrating analogs (such as C2) can induce necrosis in hepatocytes, renal, prostate, and glioma cells [43,44]. Interestingly, cell proliferation status may determine mode of death upon ceramide: stimulation of T lymphocytes by phytohemagglutinin switched necrotic program to apoptotic [45].

Redox signaling pathways

Increased production of ROS and reactive nitrogen species can be caused in organisms by macrophages during immunological response, by mitochondria, and by some other mechanisms. As a consequence of high toxicity of oxygen, aerobic cells have a number of antioxidative defense systems, and modulation of these systems has a dramatic effect on form and intensity of cell death.

Hydrogen peroxide, a component of ROS, is often used as a model reagent since it is produced as a factor of

immune defense and during various stresses. It can cause both apoptosis and necrosis of cells [46,47,48], which can be prevented by the antioxidants glutathione or *N*-acetylcystein (NAC) [23]. Surprisingly, when applied together with antitumor drugs, subtoxic doses of H₂O₂ can switch cell suicide to necrosis [48,49]. This effect was probably associated with specific signaling role of H₂O₂ rather than with inhibition of caspases or PARP activation (see below) since much higher concentration of H₂O₂ was necessary for the latter effect [47,48]. A decrease in cellular content of glutathione can also switch a form of cell death induced by ROS. For example, apoptosis of U937 tumor cells induced by Cd²⁺, cisplatin, and melphalan was switched to necrosis when glutathione synthesis was inhibited [50,51]. Interestingly, ROS-induced necrosis can also be modulated by cell transformation. Transformation of 3T3 cells by SV40-T antigen promoted menadione-induced necrosis, which can be inhibited, surprisingly, by blocking FAS receptors, although inhibitors of caspases were ineffective [52]. This result indicates that FAS receptors are involved in induction of not only caspase-dependent apoptosis, but also caspase-independent necrosis, which was also demonstrated for lymphoid cells [19].

Along with ROS, another mediator of various pathophysiological processes is NO. Because nitrosylation/denitrosylation reaction is involved in regulation of caspase-3, a key apoptotic caspase, NO may inhibit apoptosis directly through caspase-3 nitrosylation [53], although other mechanisms of inhibition of apoptosis upstream caspase-3 may also exist [54–56]. The inhibition of apoptotic pathway may be the reason why NO can switch apoptosis to necrosis upon treatment with staurosporine, ceramide, FAS, and retinoids [55,56]. NO can also bind to iron of heme-containing complexes of respiratory chain and inactivate them, potentially leading to mitochondrial damage (see “Mitochondria” section). A deleterious effect of exogenous NO can be increased by Fe²⁺ ions and by blocking GSH synthesis while NAC and SH-group donors can protect against NO. Peroxynitrite is a highly active nitrogen compound that is formed in organisms and it is often used as a model simulating action of cytokines on effector cells. Peroxynitrite formation is caused by expression of inducible NO synthase and ROS generation as a result of reaction between NO and superoxide anion. Pronecrotic effect of peroxynitrite in neuronal cells can be suppressed by NAC [57].

In most cases, antioxidants suppress both necrotic and apoptotic cell destruction. However, in 3DO hybridoma cells upon exposure to H₂O₂, NAC inhibited necrosis and stimulated apoptosis [58], while in human lymphocytes ascorbic acid activated necrosis and inhibited apoptosis [59]. It seems that oxidative stress induces an apoptotic response when cells can maintain their reducing capacity against ROS, whereas necrosis is triggered when this reducing homeostasis is disturbed (e.g., by excess of ROS or damage of natural antioxidative systems).

Protein kinases

Protein kinase JNK of MAP kinase family (also called stress-activated protein kinase, SAPK) is the major protein kinase involved in stress-induced apoptosis [60], and there are some data indicating that this kinase also participates in necrotic cell death. For instance, heat shock-induced necrosis of osteoblasts [61] and ceramide-induced necrosis in prostate carcinoma [43] correlated with JNK activation. Furthermore, inhibition of JNK decreased necrotic death of myogenic cells following transient energy deprivation [62]. Suppression of another related stress kinase, p38, reduced necrotic zone formation in the myocardium [63] and hippocampal CA1 region after ischemia [64] as well as necrosis of monocytes by toxin A of *Clostridium difficile* [9]. Ischemia/reperfusion-induced necrosis was also inhibited by expression of dominant-negative form of Rac, an upstream component of stress-signaling cascade, although this protective effect may be also related to inhibition of ROS production [65]. There is also a report about involvement of protein kinase RIP (which is associated with TNF/FAS receptors) in FAS-induced necrosis of activated T cells (see previous “Extracellular mediators, ligands, and receptors” section) [19].

On the other hand, activation of AKT kinase and MAP kinase ERK, which protect cells from stress-induced apoptosis, can protect against necrotic death as well. For example, ceramide-induced necrosis was inhibited by AKT overexpression [44]. Furthermore, AKT overexpression reduced necrotic zone formation in ischemic myocardium [66]. Accordingly, ischemia/reperfusion-induced ERK activation seems to be protective against necrosis, since its inhibition aggravated necrosis of myogenic cells in vitro [67] and myocardial infarction in vivo [68].

Therefore, it seems that proapoptotic (JNK, p38) and antiapoptotic kinases (AKT, ERK) play a similar role in necrosis. It is tempting to speculate that the common targets of these kinases in apoptosis and necrosis are mitochondria and proteins of bcl-2 family (see “Mitochondria” and “Proteins of the Bcl-2 family” sections). In addition, glutamate-induced necrosis of neuronal cells in vitro [69], and focal ischemia-induced necrosis of hippocampus in vivo was dependent on ERK activity [70].

Poly (ADP-ribose)polymerase

Poly (ADP-ribose)polymerase (PARP) is a nuclear enzyme containing a Zn-binding domain. Upon activation by DNA breaks it attaches oligomers of ADP-ribose to itself and some other nuclear proteins. Excessive activation of PARP, for example, as a result of profound induction of DNA breaks, is believed to be a cause of cell death due to ATP depletion [71,72]. This ATP depletion is resulted from use of ATP for synthesis of the PARP substrate NAD⁺. PARP inhibition (e.g., by 3-aminobenzamide and nicotinamide) suppressed cell necrosis [71] or switched it to apo-

ptosis, which was associated a marked increase in caspase activity [47,48,73].

During apoptosis, PARP is normally inactivated by caspase-specific cleavage, forming an 89-kDa fragment, a biochemical hallmark of apoptosis. If this mechanism of PARP inactivation is not operational, for example, in a PARP mutant resistant to caspase cleavage, cells become more sensitive to necrosis induced by UV radiation or TNF [74,75]. In these cells expressing mutant PARP, as well as in their wild-type counterpart, inhibition of PARP activity reduced necrosis and increased apoptosis [74]. Hence, proteolytic or pharmacological inactivation of PARP is one of the ways to prevent cell elimination through necrotic pathway. Cleavage (and, apparently, inactivation of PARP) also occurs in necrotic cells, although to fragments different from apoptotic [76]. This apparently indicates involvement of non-caspase proteases in necrosis (see “Proteases, nucleases, and phospholipases” section).

Mitochondria

Now it seems obvious that mitochondria play a crucial role in determination of cell fate under stresses. First, as a source of ATP, mitochondria chose between ATP-dependent or -independent programs. Second, as a source of tanatogenic (death-promoting) factors, mitochondria initiate or amplify the caspase-dependent apoptotic program (mainly through efflux cytochrome *c*) or activate directly the execution phase (through efflux of apoptosis induction factor, AIF). Finally, they generate ROS that also control form of cell suicide (see previous “Redox signaling pathways” section) (see Ref. [77] for review).

Apparently, maintenance of certain levels of ATP is required for execution of apoptotic programs. ATP or its derivate, dATP, is a cofactor of apoptosome [78], a high-molecular-weight complex consisting of APAF-1 and caspase-9 [79], which activates a major execution caspase, caspase-3. Besides apoptosome, ATP also seems necessary at other stages of the apoptotic program [56,80]. Generally, if the amount of ATP drops below some critical levels, this either can switch apoptotic cell death to necrotic (e.g., if cells exposed to genotoxic stress causing profound PARP activation, see previous section) or may cause necrosis by itself. Indeed, mitochondrial inhibitors can cause necrosis. Inhibitors of complex I of respiratory chain, such as rotenone, 1-methyl-4-phenylpyridium, or 6-hydroxytryptamine, which simulate cell loss during Parkinson’s disease, caused necrosis of PC12 neuroblastoma cells [81,82]. Furthermore, inhibitors of complex II, 3-nitropropionic acid, or complex III, anthimycin A, also induced necrosis [83,84]. Mitochondrial inhibitors, however, do not affect viability of cells with high level of glycolysis (e.g., tumor cells), which are capable of maintaining ATP levels without any respiration (see, e.g., [85,86]). Of note also is that drastic ATP depletion (below 3% to 5% of initial) for many hours resulted from hypoxia or starvation is not toxic for some cells (e.g.,

fibroblasts), whereas other cells (e.g., neuronal and cardiac cells) rapidly die via necrosis (see Ref. [85] for review). The reason for such different sensitivity of cells to ATP depletion is not clear, but may be associated with much more severe ionic imbalance (in particular, Ca^{2+} imbalance) in sensitive cells (see previous Ion channels and lipids section). On the other hand, necrosis can be induced in the cells with a normal amount of ATP (e.g., necrosis of AKR-2B fibroblasts under serum withdrawal [87] or TNF-induced necrosis of L929 fibrosarcoma cells [88]). This indicates that, although the ATP level may control mode of cell death, there are other factors that contribute in final outcome.

One such factor may be ROS produced by the mitochondrial respiratory chain, and this ROS generation may trigger a necrotic program [89,90]. It was hypothesized that when cellular antioxidative defense is limited, ROS caused oxidation of the key molecules and release of executor proteases, lipases, and nucleases from mitochondria [90]. The emergence of such dangerous mitochondria triggers the cell’s protective response in the form of autophagia with participation of caspases [90,91]. This hypothesis may explain why in some cells inhibition of caspases, while inhibiting TNF-induced apoptosis, may trigger necrotic cell death (see “Proteases, nucleases, and phospholipases” section). Indeed, TNF may activate mitochondrial ROS generation, and such dangerous ROS-producing mitochondria are normally eliminated by caspase-dependent autophagia [90]. However, when caspases are inhibited, these mitochondria may trigger necrotic death of a whole cell. Interestingly, because some viruses encode caspase inhibitors to avoid apoptosis of infected cells, the ability to trigger necrosis when caspases are inhibited may be an important part of the cellular antiviral defence [92]. Being the source of apoptogenic factors (cytochrome *c*, Smac/Diablo, AIF), in addition to ROS, mitochondria can be the source of pronecrotic factors as well. Under some conditions (e.g., high Ca^{2+} , oxidative stress) mitochondria undergo drastic changes accompanied by deenergization of the inner membrane, swelling, and permeabilization, a process called mitochondrial permeability transition (MPT) [93,94]. This is usually an irreversible process leading to mitochondrial “death” (mitochondrial apoptosis or “mitoptosis” [95,96]). Although it is still a matter of debate whether MPT is necessary for cytochrome *c* release and apoptotic cell death, at least in some cases blockade of MPT by specific drugs (e.g., cyclosporine, bongkrecic acid) can drastically reduce apoptosis without increasing necrosis [97,98]. It was suggested that MPT may be also an inductor of necrotic cell death through release of some mitochondrial factors (e.g., Ca^{2+} , proteases, lipases) [93,94]. Indeed, inhibitors of MPT may protect from necrosis caused by oxidative stress, hypoxia–reoxygenation in vitro [99], or ischemia–reperfusion in vivo [100,101].

Therefore, mitochondria may be the source of three relatively independent lethal signals that trigger or switch cell death pathways: ATP, ROS, and apoptogenic/necrogenic

factors. The final outcome of cell suicide is apparently dependent on interplay between these factors.

Proteins of the Bcl-2 family

Proteins of the Bcl-2 family play a very significant role in the determination of cell sensitivity to lethal signals. Antiapoptotic members of this family (Bcl-2, Bcl-X_L, etc.) can inhibit not only apoptotic, but also necrotic death. They delay or prevent necrosis evoked, for instance, by chemical anoxia [102], myocardial ischemia [103], β -amyloid [104], staurosporine with rotenone [105], or a combination of cytokines [22]. A balance between the necrotic and apoptotic cell response may also depend on a balance between pro- and antiapoptotic members of the Bcl-2 family. For instance, the antinecrotic effect of chronic hyperglycemia consists in activation of Bcl-2 expression and phosphorylation of the proapoptotic protein Bad [106]. Increased expression of Bax stimulated apoptosis, but coexpression of Bcl-X_L, surprisingly, switched cell death to necrosis [107]. It should be noted, however, that not all necrotic programs are suppressed by proteins of the Bcl-2 family, for example, necrosis caused by peroxynitrite [108] or the mitochondrial uncoupler 3-acetylpyridine [109].

A protein from the Bcl-2 family, BNIP3, which causes specifically necrotic cell death, has been recently discovered [110]. Pronecrotic functions of this protein in transfected cells are manifested by earlier plasma membrane permeabilization, cytoplasm vacuolization, and autophagy of mitochondria. These morphological changes were accompanied by mitochondrial depolarization and ROS generation and were blocked by inhibitors of MPT CsA and bongkrekic acids [110].

The main antiapoptotic and antinecrotic effect of Bcl-2/Bcl-xL proteins is believed to consist in preservation of mitochondrial integrity (i.e., prevention of MPT, efflux of cytochrome *c*, and other proapoptotic/pronecrotic factors). However, the molecular mechanisms of their effect remained yet to be unraveled.

Heat shock proteins

Heat shock proteins (Hsps) are other important regulators of lethal programs. The most studied of them are Hsp70 and Hsp27, which can inhibit apoptosis caused by various stimuli (heat shock, oxidative stress, ischemia/reperfusion, TNF, UV, anticancer drugs, and others) ([111,112]). Their overexpression also protects cells from necrosis caused by heat shock [113,114], oxidative stress [115], NO [116], and ischemia/reperfusion [85,117]. For instance, after myocardial ischemia/reperfusion, transgenic mice overexpressing Hsp70 in the heart had a smaller infarct zone, a lower level of creatine kinase (indicator of necrosis) in blood plasma, and better recovery of mechanical function [117–120]. Hsp70 is also involved in protection of brain from ischemic damage [121]. Small Hsps, Hsp27 and its homolog α B-

crystallin, can also protect cardiomyocytes from ischemia-induced necrosis in vitro and in vivo [122,123].

The protective effect of Hsp70 in myocardial ischemia was not associated with preservation of ATP level during ischemia, but ATP recovery in the myocardium of Hsp70-expressing animals was faster and higher than in control [120]. These data may indicate that Hsp70 preserve mitochondrial functions during ischemia/reperfusion and/or accelerate the recovery of these functions. Similar effects were observed upon expression of mitochondrial chaperones Hsp60 and Hsp10 in cardiomyocytes: protection of cells from ischemia correlated with preservation of mitochondrial complexes III and IV activity and better ATP recovery [124]. Furthermore, the protective effect of Hsp70 against NO-induced necrosis in human β -cells was not associated with suppression of lipid peroxidation, but also with rescue of mitochondrial functions (tetrazolium reduction) [125]. However, since neither Hsp70 nor Hsp27 are localized to mitochondria, it seems unlikely that their protective action is associated with direct effect on mitochondrial structure. Probably, these chaperones suppress signal transduction pathways leading to mitochondrial damage and cell death. These pathways may include the stress kinases JNK and p38 (see previous “Protein Kinases” section). Indeed, activation of these kinases was markedly increased during ischemia/reperfusion, and their inhibition suppressed necrosis in vitro and in vivo [63,126]. Because activation of JNK and p38 after in vitro “ischemia” of myogenic cells was reduced in Hsp70-expressing cells [62], these kinases may be the targets of antinecrotic effect of Hsp70 in the myocardium. Interestingly, protection of the kidney from ischemia/reperfusion by ischemic preconditioning was also associated with stress kinase suppression, although in this case it was Hsp27 rather than Hsp70 that was accumulated in the preconditioned kidney [127].

Therefore, the molecular chaperones Hsp70 and Hsp27, along with proteins of the Bcl-2 family, are powerful inhibitors of necrosis. It seems that, although they have quite different mechanism of action, the main targets of their protective effect are mitochondria, either directly (in case of Bcl-2/Bcl-xL) or indirectly (in case of Hsp70/Hsp27).

Proteases, nucleases, and phospholipases

Proteolytic enzymes perform crucial functions in suicide elimination of cells: transduction of lethal signal via cascades of caspase and a final destruction of various protein targets (PARP, lamins, cytoskeletal proteins). Cysteine proteases of the caspase family play the key role in these processes [128]. In many models it is the only way of execution of apoptosis, because in the presence of endogenous or exogenous caspase inhibitors or in the absence of caspase expression, suicidal programs are completely blocked or switched to a necrotic pathway. For instance, caspase inhibitors switched to necrosis cell death caused by irradiation, camphotechine, etoposide, dexametasone, in-

ductors of MPT, and activation of purine receptors [129–132]. Inhibition of caspase-3/7 by exogenous NO switches apoptosis to necrosis despite efflux of cytochrome *c* from mitochondria [56]. LCC human carcinoma cells deficient in caspases died via necrosis in the presence of a zinc chelator, while caspase-expressing cells died via apoptosis [133]. As mentioned previously (“Physiological and pathophysiological stimuli leading to necrosis” section), in mice without Apaf-1 or caspase-3/caspase-9, apoptotic cell death during development switched to necrotic [134].

Surprisingly, there are some models where caspase inhibition not only prevented apoptosis but also severely aggravated necrosis. In L929 fibrosarcoma cells, inhibition of caspases increased the cell’s sensitivity to TNF-induced necrosis by a factor of 1000 [135]. The similar effect was observed during excitotoxic death of hippocampal neurons [136]. These data indicate that caspases may also play an antinecrotic role consisting of elimination of “harmful” mitochondria that produce high level of ROS, and if such mitochondrial killing fails, necrosis is triggered (see previous “Mitochondria” section).

However, in some circumstances the execution of the necrotic program requires caspase activation. Necrotic death caused by ATP depletion in CD95-stimulated Jurkat cells was suppressed by the pancaspase inhibitor z-VAD.fmk [137]. This inhibitor (but not z-DEVD.fmk, an inhibitor of caspase-3) also reduced TNF-induced necrosis in enterocytes [138] and fibrosarcoma cells [139]. Inhibition of caspases also prevented necrosis caused by toxin A of *C. difficile*, toxin α of *Staphylococcus aureus*; ouabain, or nigricine [140].

During the past years, a number of data emerged demonstrating wide occurrence of caspase-independent programmed cell death, both apoptotic and necrotic [141,142]. For instance, TNF-induced cell death of hepatocytes and tumor cells apparently requires lysosomal cysteine protease cathepsin B [143,144]. Another cysteine protease, Ca^{2+} -dependent calpain, may participate in ischemia-induced cell death of hepatocytes after ischemia/reperfusion [145]. Inhibitors of calpain suppressed Ca^{2+} -induced necrosis of neurons [146,147] or switched NMDA-induced death of these cells from necrosis to apoptosis. Presenilin-1, a transmembrane protein that proteolytically processes β -amyloid protein in Alzheimer’s disease, apparently participates in protection from excitotoxic death of neurons, since transfection of mutant protein increased their sensitivity to necrosis [148]. Finally, some as yet unidentified serine proteases can participate in TNF-induced necrosis of L929 cells [25] and necrosis of kidney cells induced by “chemical hypoxia” [10].

Therefore, the role of caspases, key executor caspases in apoptosis, is more diverse in necrosis. Their inhibition may either suppress or activate necrosis depending on cell line and stimuli. It is probable that switching from apoptosis to necrosis in the presence of caspase inhibitors, at least in some cases, may be associated with ATP depletion due to

PARP activation (see previous “Poly(ADP-ribose)polymerase” section). If caspase inhibition prevents caspase-dependent PARP inactivation, it may cause ATP depletion, blockade of ATP-dependent apoptosis, and triggering of necrosis. At present, however, little is known how caspases participate in necrosis, but they may play signaling role in activation of other proteases such as cathepsin, calpain, and serine proteases whose involvement in execution of necrosis was demonstrated in some circumstances.

Along with proteolysis, necrosis is also accompanied by degradation of DNA. Degradation of DNA during necrosis usually occurs randomly, forming a “smear” pattern on agarose gels, while apoptotic DNA fragmentation occurs to oligonucleosome fragments forming a remarkable “ladder” pattern on the gels. The main apoptotic nuclease is CAD (caspase-activated DNase), whereas caspase-independent DNase I and II are probably implicated in necrosis. For instance, an increase in DNase I-like endonuclease activity was observed in the kidney cortex after ischemia/reperfusion [149], and activation of DNase II was found in the necrotic hippocampus after global ischemia [150]. However, the mechanisms of activation of these nucleases presently are not known.

Activation of some phospholipases during necrosis, especially cytosolic Ca^{2+} -dependent phospholipase A2 (cPLA2), has been also demonstrated (see Ref. [151] for review). Activity of cPLA2 was increased in hippocampal slices immediately following exposure to ischemic conditions, and this enhancement lasted for at least 24 h; furthermore, pharmacological blockade of cPLA2 (by bromophenacyl bromide or AACOCF3) prevented neuronal death [152]. Likewise, TNF-induced necrosis of MCF7 cells was suppressed by cPLA2 inhibitors [153]. In contrast to necrosis, cPLA2 activity was dispensable for TNF-induced apoptosis of HeLa cells; moreover, during apoptosis cPLA2 underwent caspase-dependent cleavage and inactivation [154]. Such inactivation of cPLA2 during apoptosis may represent a mechanism to avoid the inflammatory response against apoptotic cells that may be evoked by products of phospholipid hydrolysis.

Molecular scenario of necrotic cell death

The data described in the previous sections can suggest a possible molecular scenario of necrosis (Fig. 1). There are several receptors implicated in triggering necrosis; among them are TNF receptors and other receptors of these family (FAS, TRAIL), purinogenic receptors, and excitoreceptors (e.g., NMDA). Another important sensor is DNA: its damage may be induced either directly (e.g., by radiation or anticancer drugs) or indirectly through oxidative stress (e.g., upon ischemia/reperfusion or other ROS generating treatments). Massive DNA breaks may cause activation of PARP, depleting its substrate NAD^+ and, subsequently, ATP, which may lead to necrosis due to energy deficiency.

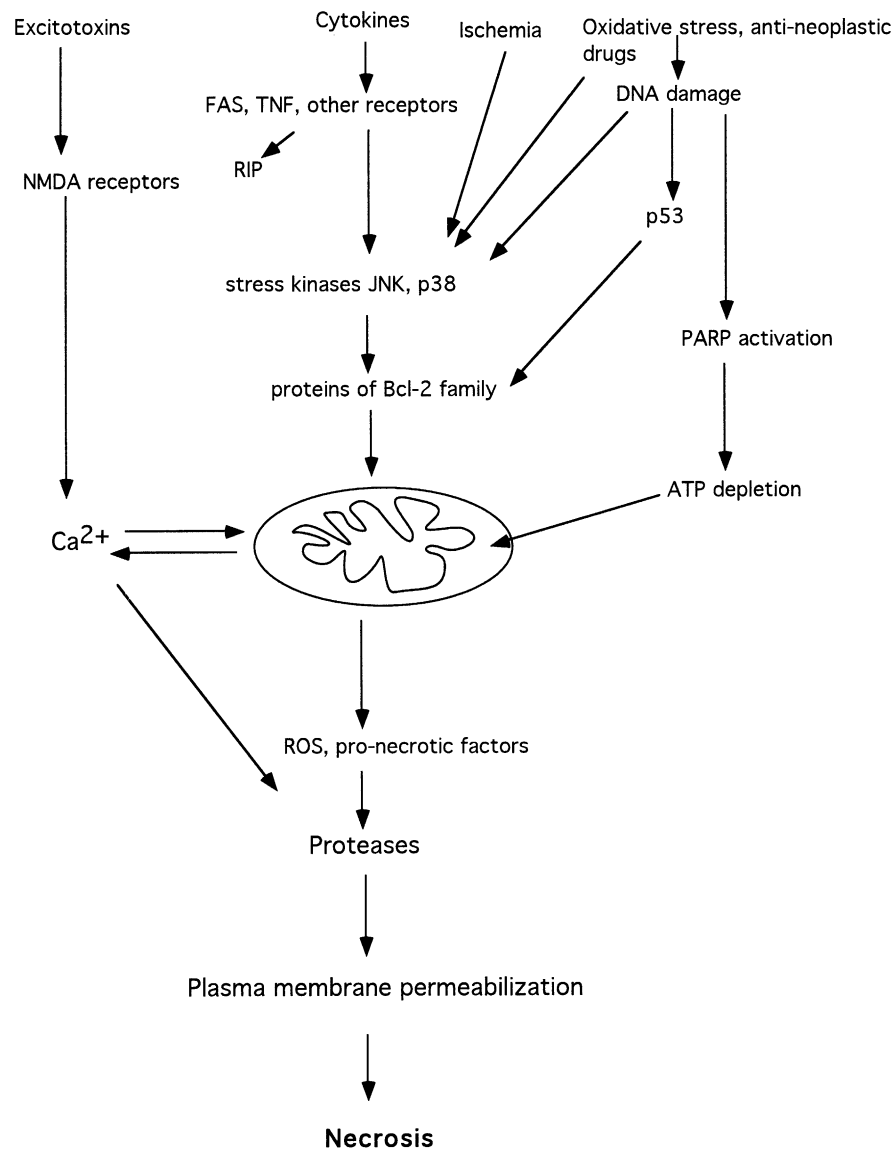


Fig. 1. A possible molecular scenario of necrosis. See text for further explanations.

Among the second messengers participating in receptor-mediated necrosis are Ca^{2+} and ceramide. Blockade of Ca^{2+} influx or buffering of its efflux from intracellular depots can prevent necrosis caused by excitotoxins. Ceramide accumulation may be involved in some cases of necrosis, although its role in cell death as a messenger is still a matter of debate, because it can accumulate just as a consequence of cell death. Stimulation of the receptors, oxidative stress, and DNA damage are powerful activators of stress kinases of JNK and p38, which are apparently common components of both apoptotic and necrotic programs. At present it is not clear why their activation can lead to apoptosis in some cases and necrosis in others, but it is tempting to speculate that the extent of mitochondrial damage evoked by the activation of these kinases may determine cell fate. Indeed, mitochondria, besides their role in ATP generation along with glycolysis, obviously play the key

role in determination of a pathway of cell suicide. Mitochondria are powerful sources of tanathogenic factors such as cytochrome *c*, AIF, and ROS, and they are the main targets of cell survival systems (proteins of the Bcl-2 family, heat shock proteins). The amount of ATP may be the essential factor that determines the choice of the cell suicide pathway, but there are obviously other important (but yet unknown) factors.

Finally, the last stage of necrotic destruction is the activation of proteases. In several models of necrosis, this destruction is executed by caspases, but in many cases inhibition of caspases during stresses may trigger necrosis rather than suppress it. This indicates that caspase activity is sometimes necessary, paradoxically, for protection of cells from stresses, possibly through caspase-mediated elimination of ROS-generating mitochondria. Among proteases probably involved in necrotic digestion are calpains, cathe-

psins, and serine proteases, but their cellular targets in necrotic cell destruction are yet to be elucidated.

Necrotic death is a regulated cellular response to stress and its physiological consequences

The conception of programmed cell death is based on the resemblance of biochemical mechanisms of cell demise and includes not only processes of cell elimination, but also terminal differentiation (e.g., in keratinocytes and reticulocytes). An important component of PCD is apparently “mitotic” or “reproductive” cell death, that is, death of damaged but mitotically active cells after several divisions. This cell death can also be called “accelerated aging” because a cell cannot divide a genetically determined number of times and die prematurely, via necrosis or otherwise. The phenomenon of limited cell division of normal (untransformed) cells is known as the Hayflick limit [155]. Interestingly, there was a correlation between necrosis and other indexes of mitotic cell death (number of micronuclei and loss of colony forming ability) [156,157]. Necrotic cell death as a form of PCD allows us to consider elimination of “unwanted” cells regarding pathophysiological consequences of final stages of cell’s destruction: what suicidal programs *in vivo* may result in efflux of cellular constituents in extracellular space and what programs block this process either through maintaining plasma membrane integrity and/or provoking phagocytosis.

Generally, stressful stimuli can initiate programs, which in a reversible form culminate in cell proliferation, differentiation, or senescence (Fig. 2). In an irreversible phase (phase of choice of cell suicide), a cellular thanatogenic mechanism usually triggers the apoptotic form of cell destruction using caspase-dependent and -independent pathways to avoid inflammatory and autoimmune reactions that are potentially dangerous for an organism (Fig. 2). However, in some cases the necrotic pathway is triggered, and triggering necrosis instead of apoptosis is not just a cell’s failure, but may have a positive effect when a strong inflammatory response is necessary. Indeed, there are indications that choice of program of autodestruction occurs before initiation of the irreversible phase of cell response to a lethal signal. The hallmark of apoptosis, externalization of phosphatidylserine, that designates a cell with an “eat me” message, is the earliest feature of apoptosis triggering [158,159]. However, in necrotic cells this feature is usually registered after plasma membrane destruction; therefore, necrotizing cells are not recognized by phagocytes and they cannot be digested until their intracellular contents are spilled into the extracellular space [160].

The question arises: what signals does the immune system receive from necrotic cells? Some of the signals are already known; among them are Hsp70, calreticuline, oligonucleosomes, and carbohydrates [161]. When delivered in the extracellular space, these substances activate antigen-

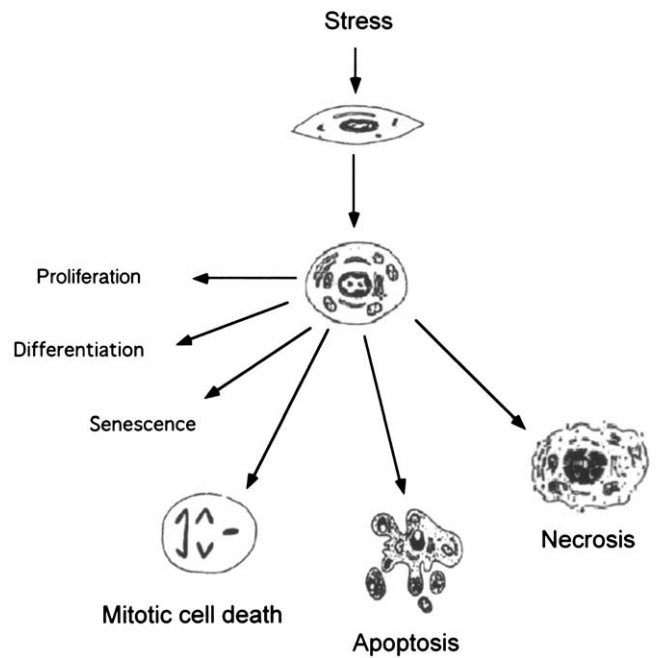


Fig. 2. Necrosis is a regulated cellular response to stress. Depending on cell type and extracellular conditions, type and intensity of stressful stimuli, and other factors, there may be several responses of cells to stress, and necrotic cell death is one of them. See text for further explanations.

presenting cells (APC) including dendritic cells [161]. The most important role is attributed to Hsp70 [162,163]. Its elevated levels in necrotic neoplastic cells markedly increased their immunogenicity by promoting the Th1 response and APC maturation [164]. Thus, Hsp70 is not only a marker of necrosis, but also a specific signal for the immune system. Hsp70 has high immunogenicity by itself and increases immunogenicity of some other macromolecular antigens [163]. Furthermore, exogenous Hsp70 can elicit production of proinflammatory cytokines in monocytes via activation of CD14 receptors [165].

Depending on molecular signals from necrotic cells (which are alien to surrounding cells), diverse type of these cells (neutrophils, macrophages, and others) become involved in the immune response. It was found that necrotic cells are more efficient than apoptotic cells in their capacity to stimulate the APC and T-cell response [164]. On the other hand, apoptotic cells induced in APC the secretion of cytokines that inhibit Th1 response [166]. Necrotizing tumor cells also potentiate maturation of dendritic cells and optimal presentation of tumor antigens [164,167]. These data indicate that a much more robust immune response is evoked during necrosis than from apoptosis. This may be physiologically important during some dangerous situations such as viral or bacterial infection, trauma, and abnormal (transformed) cells, when strong stimuli produced by necrosis are required for mobilization of all cell defense forces (dendritic cells, monocytes, and neutrophils). Indeed, although some viruses encode caspase inhibitors to avoid apoptosis of host cells, the ability of these cells to activate

the necrotic pathway of cell suicide is apparently of adaptive significance. This response, however, may become a chronic chain reaction of inflammation; therefore, necrotic cell death occurs in an organism only in relatively rare cases.

Modulation of necrosis for therapeutic purposes

The accumulating data indicate that necrotic cell death, being a regulated form of cell demise, can be modulated for therapeutic purposes: it may be suppressed to prevent damage of normal tissues or activated to induce damage of tumor tissues. Ischemic conditions are the conditions when prevention of necrosis may be of a great importance. Although inhibitors of caspases alleviated apoptosis and reduce ischemic damage in the myocardium and brain [168,169], caspase inhibition per se may simply switch modes of cell death without increasing overall cell survival (see previous “Proteases, nucleases, and phospholipases” section). Moreover, the efficiency of caspase inhibitors in reducing necrosis may also be due to their anti-inflammatory properties [170,171]. It seems more appropriate to block both cell death programs, apoptosis and necrosis, to achieve maximal cell survival. To do this, some upstream components of signal transduction pathways of both programs should be inactivated. Stress kinases p38 and JNK are among such components. Indeed, inhibition of p38 by its specific inhibitor SB203580 was shown to reduce infarct size following ischemia/reperfusion [63]. In a model of brain ischemia, the p38 inhibitor SB203580 reduced cell death following a transient global ischemia in the most sensitive CA1 region [64]. There is also a report that administration of U0126, an inhibitor of MEK, an upstream component of ERK cascade, protected the hippocampus against forebrain ischemia [70].

The search for effective drugs for induction of heat shock proteins (Hsp70, Hsp27) may be another promising approach to block both apoptotic and necrotic damage during ischemia of the myocardium and brain. Indeed, accumulation of these proteins after heat shock treatment or their delivery to these organs by viral vectors significantly reduced damage and cell death [172]. Other inhibitors of ischemic damage may be PARP inhibitors and inhibitors of mitochondrial damage. PARP inhibitor (3-aminobezamide) was effective in prevention of myocardial [173,174] and cerebral [175,176] damage following ischemia, and this effect was not associated with inhibition of apoptotic component of cell death [175,177]. Furthermore, cyclosporine A (an inhibitor of mitochondrial pore opening, see previous “Mitochondria” section) reduced necrosis of the myocardium and brain [178,179].

In a streptozotocin-induced experimental model of diabetes, pancreatic cells die via apoptosis and necrosis. It is probable that release of highly immunogenic proteins from necrotizing cells in the extracellular space provokes inflammation and cell destruction, typical signs in pathogenesis of

diabetes. Therefore, prevention of necrosis of β -cells may be helpful in therapy of the disease. PARP inhibitors [180,181] and inhibitors of NO synthase [22,182] were found to be effective in this model. Although inhibitors of PARP cannot suppress apoptosis, prevention of release of intracellular components should decrease inflammatory response.

An important component of pathogenesis of Alzheimer’s disease is formation of β -amyloid, which can cause neuronal cell death. Necrosis of PC12 neuronal cell culture by this protein was prevented by increasing cyclic GMP accumulation either by a phosphodiesterase inhibitor (e.g., propentophylline) or by NO^{*} generation (by nitrosoacetylpenicillamine, SNAP) [183].

Suppression of necrosis of cells infected with intracellular pathogens may be sometimes helpful in prevention of invasion of pathogens and escalation of infection, while stimulation of apoptosis may increase the efficiency of pathogen eradication. Inhibitors of Ca²⁺ channel verapamil inhibit necrosis of endotheliocytes infected with rotavirus [38]. On the other hand, fast necrosis of infected cells may prevent intracellular multiplication and accumulation of pathogens and cause a strong immune response (see previous “Necrotic death is a regulated cellular response to stress and its physiological consequences” section), so application of inhibitors of necrosis to fight infection may depend on many factors that should be carefully studied.

Despite numerous efforts, there is a little progress in increasing the efficiency of antitumor therapy by induction of apoptosis of neoplastic cells. The rate of apoptosis shows a little correlation with suppression of clonogenic ability of cells, which results in tumor recurrence [184,185]. This situation led to a search for treatments activating the proinflammatory response to antitumor therapy. Such activation has been achieved by induction of necrosis. For instance, introduction in tumor cells the suicide gene of thymidine kinase in combination with gancyclovir caused necrosis of murine B16 tumor cells, which was accompanied by a high antitumor immune response of the Th1 type [186]. Another approach may be blocking of phagocytosis of apoptotic cells that should lead to their “secondary” necrosis and release of proinflammatory substances. One such method may be application of substances that imitate externalization of phagocytosis markers (e.g., liposomes with phosphatidyl serine or soluble phospho-L-serine) [187]. Some treatments currently used in cancer therapy, along with apoptosis, can cause necrosis of tumor cells. These are gamma irradiation [129,188], photodynamic therapy [189,190], doxorubicin [191], docetaxel [184], and phenitidine (a retinol derivative) [192]. Interestingly, mutant cells with increased sensitivity to some agents die via a necrotic mode, for example, ATM mutant upon gamma irradiation [157] or Fanconi mutant upon mitomycin treatment [193]. Therefore, a perspective strategy to increase efficiency of anticancer therapy is treatments (or their combinations) that promote the necrotic form of cell death.

Conclusion

For a long time necrosis was considered as an alternative to a programmed cell death, apoptosis. However, recent data indicate that there are several examples when this form of cell death may be a normal physiological and programmed event (e.g., during tissue renewal, embryogenesis, and immune response). Therefore, necrosis, along with apoptosis, may be considered as a form of the execution phase of programmed cell death. However, the consequences of necrotic and apoptotic cell death are quite different for a whole organism. In the case of necrosis, cytosolic constituents that pour into the intercellular space through the damaged plasma membrane may provoke the inflammatory response; during apoptosis these products are safely isolated inside macrophages. The inflammatory response caused by necrosis, however, may have obvious adaptive significance (i.e., emergence of a strong immune response) under some pathological conditions (e.g., cancer and infection), and disturbance of a fine balance between necrosis and apoptosis may be a key element in development of some diseases.

Acknowledgments

This work has been supported in part by Russian Academy of Medical Sciences, the Russian Foundation of Basic Research (Grant 01-04-4942), the American Heart Association (to V.G.), and the International Science and Technology Center (Project 779B), Moscow, Russia. The authors are grateful to Prof. V.P. Skulachev (A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University), Prof. E.F. Luschnikov (Medical Radiological Research Center, Obninsk), and Prof. M.Y. Sherman (Boston University Medical School) for critical reading of the manuscript.

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