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**REVIEW**

# Necrosis Is an Active and Controlled Form of Programmed Cell Death

**S. Ya. Proskuryakov[1]*, V. L. Gabai[2], and A. G. Konoplyannikov[1]**

*[1]Medical Radiological Research Center, Russian Academy of Medical Sciences, Obninsk, 249020 Russia; fax: +7 (095) 956-1440; E-mail: noo@mrrc.obninsk.ru*
*[2]Department of Biochemistry, Boston University School of Medicine, Boston, USA; fax: (617) 638-5339; E-mail: gabai@biochem.bumc.bu.edu*

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**Abstract**—In all studies on programmed cell death (PCD) and apoptosis as its most showy form, this process was considered to be a paradigmatic antithesis to necrotic cell death. On one hand, a concept on necrosis as a cellular cataclysm, an uncontrolled and passive phenomenon, had been provoked by an enormous bulk of experimental data on its inducibility by superphysiological exposures. On the other hand, much attention was attracted to a rapidly expanding (from nematodes) field of genetic studies on PCD. However, the findings accumulated which suggested a likeness rather than the opposition of the necrotic and apoptotic forms of elimination of "unwanted" cells. 1. Very diverse pathophysiological exposures (stimuli, stresses), such as heat, ionizing radiation, pathogens, cytokines cause both forms of cell death in the same cell population. 2. Antiapoptotic mechanisms (e.g., Bcl-2) can protect cells from both necrotic and apoptotic destruction. 3. Biochemical interventions (e.g., with inhibitors of poly-(ADP-riboso)-polymerase) into the signal and executive mechanisms of PCD can change the choice of the cell death form. 4. During both necrosis and epigenetic programs of apoptotic cell death that need no macromolecular synthesis (e.g., the CD95-dependent death), the nucleus plays a passive role. Therefore, necrosis, similarly to apoptosis, is suggested to be a form of the programmed cell death. However, for the whole body the physiological consequences of apoptosis and necrosis are quite different. In the case of apoptosis, all constituents of the nucleus and cytoplasm are isolated by an undamaged membrane and then by phagocytes together with the membrane-bound "eat me" markers (phosphatidylserine, etc.). In other words, the elimination of the cell which has realized its apoptotic program remains virtually unnoticed by the body. In the case of necrosis, the cytoplasmic content released into the intercellular space provokes an inflammatory response, i.e., an activation of resident phagocytes and attraction of leukocytes into the necrosis zone. It is suggested that under pathophysiological conditions, the necrotic cell destruction should amplify and catalyze pathological processes. The experimental data available now suggest that a disturbance in the body of optimal balance between the necrotic and apoptotic forms of PCD should be a crucial factor in the development of various pathophysiological processes associated with inflammation (diabetes, arthritis) or with aging (atherosclerosis, neurodegenerative diseases).

*Key words*: necrotic form of programmed cell death, inhibitors, inducers, and modulators of necrosis, molecular mechanisms

## 1. PROGRAMS OF CELL DEATH: NECROSIS AND APOPTOSIS

In the early 1970s, an increasing amount of morphological data in favor of a new phenomenon in cell death was summarized as the concept on the apoptotic form of cell destruction [1]. Such a denomination (which was used even in the Ancient Greece medicine [2]) of this process emphasized its resemblance to a natural physiological phenomena (from the Greek αποπτωσιζ that means a leaf fall). The necrotic cell death as a prototype retained the phenomena which seemed to find no place in the new process associated with morphological and biochemical events regularly and predictably follow-

*Abbreviations*: ARC) antigen-representing cells; ROS) reactive oxygen species ($OH^{\cdot}$, $O_2^{\cdot-}$, $H_2O_2$); RNS) reactive nitrogen species ($NO^{\cdot}$, $NO_2^{\cdot}$, $ONOO^-$); IR) ionizing radiation; I/R) ischemia/reperfusion; SNP) sodium nitroprusside; PCD) programmed cell death; MMP) mitochondrial membrane permeability; LPO) lipid peroxidation; PDT) photodynamic therapy; PS) phosphatidylserine; cyt. C) cytochrome C; CR) ceramide; Casp) caspase; cGMP) cyclic guanosine monophosphate; Hsp) heat shock proteins; LDL) low density lipids; MAPK) mitogen-activated protein kinases; i-NOS) inducible nitric oxide synthase; LPS) lipopolysaccharide; NAC) N-acetylcysteine; NA) nicotinamide; PARP) poly-(ADP-riboso)-polymerase.

* To whom correspondence should be addressed.

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ing each other and nearly independent of stress stimulus [3].

The development of the apoptosis concept was associated with identification of biochemical reactions associated with morphological changes [4]. However, later this concept was significantly extended by a hypothesis on programmed cell death (PCD). One of the main conclusions from this hypothesis was the idea that the cell death induced by pathophysiological stimuli is a particular case of realization of an evolutionary conservative mechanism responsible for cell elimination under the influence of morphogenetic and/or homeostatic signals in both animal and plant cells [5-8].

A new stage in the striking success of the PCD theory was marked by the discovery of a genetic apparatus responsible for switching and functions of biochemical mechanisms of animal cell destruction [9].

This seems to be the reason for insufficient attention given to reports about the existence of epigenetic mechanisms of cell death which needed no gene expression and activation of macromolecular synthesis [10, 11]. In 1989 one of such mechanisms, the destruction of human lymphoid cells under the influence of certain antibodies, was described and denoted as a CD95-dependent cell death [12]. It was especially interesting that specific apoptotic changes were induced by such processes even in cytoplasts, i.e., it needed no nucleus [13, 14]. These data on the presence in cells of a constitutive thanatogenous (the Greek *thanatos* means death) apparatus had started the weakening of a dogmatic antithesis between apoptosis and necrosis because the latter seemed to have no need for gene expression [15-18]. In the framework of the analysis performed, we shall have in mind but neglect the discussion about essential difference in the last stages of the apoptotic and necrotic cell death *in vivo* and *in vitro* [19, 20].

The purpose of the present work was to consider molecular mechanisms of necrotic cell death characterized by disturbance of the plasma membrane integrity caused by physiological or close to physiological exposures (but not by superphysiological!) and also possibilities to control these mechanisms.

## 2. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STIMULI OF NECROTIC CELL DEATH

Superphysiological exposures (e.g., a thermal or acid burn) of tissues (cells) obviously result in an uncontrolled destruction of cell membranes, of cyto- and caryoarchitecture, etc. This observation induces a general opinion that disorders in the plasma membrane integrity and in the cytoplasm and nucleus structures which characterize the cell destruction also suggest that necrosis should be a passive and uncontrolled process [21-27]. However, this conventional conclusion conceals many contradicting phenomena (Table 1).

Approaches developed in cytology have shown that necrotic death is a normal element of the body life activities: embryogenesis, ovogenesis, and cell renewal [28-32].

**Table 1.** Necrotic death during physiological and pathophysiological processes

| Process | Object/stimulus | References |
|---|---|---|
| Embryogenesis | Neurons | [15, 29] |
| Ovogenesis | Follicles | [31, 32] |
| Cell renewal | Large intestine epithelium | [29] |
| | Small intestine epithelium | [30] |
| Infection | Lymphocytes (HIV-1) | [55, 56] |
| | Mouse liver (*Mycobacterium avium*) | [44] |
| | Neutrophils (*Shigella flexneri*) | [57] |
| | Fibroblasts (*Trypanosoma cruzi*) | [58] |
| Diseases of the central nervous system | Alzheimer's disease | [59] |
| | Creutzfeldt–Jacob disease | [60] |
| | Epilepsy | [61] |
| Inflammatory diseases | Diabetes | [62] |
| | Syndrome of systemic inflammation | [63] |
| | Liver cirrhosis | [64, 65] |
| Ischemias | Brain | [61, 66] |
| | Heart | [67-69] |
| | Kidneys | [70] |

In model systems of cell cultures and *in vivo* the reproduction of various infectious agents, such as viruses (polyoma virus [33], rotavirus [34], parvovirus [35]), bacteria, protozoan, fungi (*Aspergillus fumigates* [36]), is accompanied by necrotic death of target cells. Such a suicidal program can be also stimulated by peptide toxins released by pathogens (the *Clostridium difficile* toxin A, the *Staphylococcus aureus* toxin α [37], streptolysin O [38]) and also by components of the animal immune system: anti-Thy-1.1 [39], the B-cell antigen receptor [40], activated natural killers [41], microglia [42], and peritoneal macrophages [43]. In the cells of the organs which have functions of the body immunological barriers (e.g., intestine, lungs) the necrotic program can be induced due to a simplified invasion of pathogens through an inflammation-injured surface [44, 45] and also (in the case of intracellular pathogens) can prevent the apoptotic death of the cell-host which in this case is a "hero" as a powerful tool in the inactivation of microorganisms [46, 47].

The death of neurons associated with various diseases of the central nervous system (Alzheimer's disease, Parkinson's disease, epilepsy, etc.) is a unique example of realization of a wide spectrum of lethal programs including necrosis [48-50]. The destruction of neurons in these cases is mainly stimulated by intensive ejection of excitotoxins [51-54].

A dramatic impoverishment of the extracellular space with oxygen, glucose, trophic factors, and acidosis are common events associated with insults, infarctions, and other states caused by thromboses of blood vessels, vasoconstriction, or trauma.

These events result in disastrous consequences, in particular, in a mass death of endotheliocytes and nonproliferating cells of the adjacent tissues: neurons, kidney cells, myocytes, etc. [67, 69]. All this occurs concurrently with phagocytosis of damaged cells and with their free destruction along with a disturbance in the plasma membrane integrity and a release of intracellular contents [26].

Diabetes, gastrites, liver cirrhosis, arthritis, and other diseases characterized by an inadequate secretion of cytokines, nitric oxide, and reactive oxygen species are accompanied by intensive losses of parenchymal cells [62, 64, 70-72]. These chronic processes seem to be maintained and stimulated by antigens excreted from necrotically dying cells.

Antitumor treatment includes genotoxic exposures which are used both alone and combined with hyperthermia, hyperglycemia, electron acceptors, and other factors [73-75]. In these cases DNA plays a role of a sensor perceiving a damaging signal of ionizing radiation (IR), photodynamic therapy (PDT), or DNA-tropic drugs. But the realization pathways of the lethal program in tumor cells depend on the exposure dose, microenvironmental factors (the availability of oxygen and glucose, the extracellular matrix composition) and, therefore, can result in both apoptotic phagocytosis and necrotic lysis [76-80].

Thus, the elaboration of methods for differentiation of two forms of death has revealed their close coexistence in the same tissue or in the cell culture. And their biochemical and morphological features are essentially overlapped reflecting the cell possession of a wide spectrum of thanatogenous programs [17, 18, 66, 81]. Each extreme type of cell death (necrosis and apoptosis) includes quite different subprograms of destruction [39, 82, 83].

Consequently, it is reasonable to consider the necrotic cell death induced by physiological (normal) or pathophysiological influences as a form of PCD. This is indirectly confirmed by attempts to describe necrosis as a modification of apoptosis using the appropriate terms [84-86].

## 3. RECEPTORS, MEDIATORS, AND EXECUTIONERS OF NECROSIS PROGRAM

**3.1. Intercellular mediators.** Cytokines and growth factors which are released from the cells of a damaged tissue are the most important mediators of inflammation and infection. Many of these agents can induce necrotic programs [62, 87] (Table 2).

The role of various cytokines and of their combinations in the damage of secreting cells of the pancreas is intensively studied on cell and tissue models of diabetes mellitus. Table 2 shows that the necrotic response of β-cells was suppressed either by inhibition of $NO^{\bullet}$ synthesis with N-monomethyl-L-arginine (NMMA) or by expression of the antiapoptotic gene *Bcl-2*. And an increase in the fraction of necrotic cells relative to that of apoptotic ones correlated with a decrease in the activity of caspases [62].

The realization of the necrosis program also depended on the activity of mitogen-activated protein kinases (MAPK) involved in the transmission of the lethal signal. The inhibition of p38-MAPK ((i)p38-SB2033580), and ERK-MAPK ((i)ERK-PD098059) suppressed necrotic cell death and slightly stimulated apoptotic death [88].

Under conditions of diabetic damage, programs of survival are also initiated. This is associated with expression of proteins maintaining redox-homeostasis, such as Mn-superoxide dismutase, heme oxygenase, i-NOS, and Hsp70, which are components of the cell survival program [96]. Note, that unlike the case of β-cells and macrophages [95], the necrosis of cultured chondrocytes was increased on the suppression of $NO^{\bullet}$ production. However, in the presence of scavengers of reactive oxygen species (ROS), the necrosis program induced by proinflammatory cytokines was switched to the apoptotic one [71].

A very important bulk of data on the mechanism of lethal signal transmission has been obtained by studies on the thanatogenous effect of TNF-α on fibrosarcoma cell lines [81, 91, 93]. This necrosis was characterized by an

**Table 2.** Cytokines as inducers of necrosis

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| IL-1β + IFN-γ + TNF-α | Islets of rat pancreas | NMMA (–)<br>*Bcl*-2 (–) | NMMA (–)<br>*Bcl*-2 (–) | [62] |
| IL-1β + IFN-γ | Rat β-cells | {(i)p38 + (i)ERK} (–) | {(i)p38 + (i)ERK} (+) | [88] |
| IL-1β + IFN-γ + TNF-α | Mouse β-cells | i-*Nos*$^{(-/-)}$ (–) | i-*Nos*$^{(-/-)}$ (±) | [89] |
| IL-1β + TNF-α + LPS | Chondrocytes* | NMMA (+) | NMMA (±) | [71] |
| TNF-α | L929.Hcd95 | TPCK (–), TLCK (–)<br>LiCl (+), STS (+) | | [90] |
| TNF-α | Mouse fibrosarcoma (L929) | *CrmA* (+), BHA (–)<br>z-VAD.fmk (+) | z-VAD.fmk (–) | [91]<br>[81] |
| TNF-α | L929 | | AcD (+) | [92] |
| TNF-α | Mouse fibrosarcoma (WEHI-164) | z-VAD.fmk (–) | z-VAD.fmk (–) | [93] |
| TNF-α | Mouse enterocytes (IEC-6) | z-VAD.fmk (–)<br>z-DEVD.fmk (±) | | [94] |
| LPS + IFN-γ | Macrophages of C3H/OuJ mice | NMMA (–), *Irf*$^{(-/-)}$ (–) | NMMA (–) | [95] |

Notes to this and to the following tables: (–), inhibitor either of necrosis and/or apoptosis; (+), activator either of necrosis and/or apoptosis; (±), the absence of effect; *, human cells. Expressed or ($^{-/-}$) "knocked-out" genes are italicized; human genes are printed in capital letters.

early destruction of the structure of mitochondria and by their autophagy. A decrease in $pO_2$, an inhibition of mitochondrial complexes I and II of electron transmission, and also butylated hydroxyanisole (BHA) prevented the death of L929 cells. However, z-VAD.fmk (a pancaspase inhibitor) and CrmA (a protein product of a viral gene, an inhibitor of a number of caspases) increased this death tremendously. These cells transfected with a human gene of the lethal receptor *CD95* retained their sensitivity to the TNF-induced necrosis, but died apoptotically due to ligation of the receptor. Inhibitors of serine proteases suppressed the lysis, whereas lithium and staurosporine (an inhibitor of protein kinases) synergistically affected the toxicity of TNF [90].

Fibrosarcoma WEHI-164 cells were also sensitive to the lethal effect of TNF, but the destruction form depended on the stage of the cell cycle. The resting cells in the G(0)/G(1) stage displayed necrosis, whereas cells moving in the cycle displayed apoptosis. However, in this case a pancaspase inhibitor z-VAD.fmk inhibited the execution of both processes [93]. A strong lethal effect of LPS + IFN-γ on mouse macrophages is also explained by induction of TNF-α. The inhibition of p55 and NO$^{\bullet}$ mediators of this effect of the TNF-receptor suppressed both necrotic and apoptotic constituents of the process. A "knock-out" by the *Irf-1* gene (the interferon-regulated factor) resulted in the same effect [95].

Thus, intercellular mediator cytokines induced by a pathophysiological stimulus (infection, inflammation, etc.) along with other reactions activate different forms of PCD—both apoptosis and necrosis. These death programs are realized by a target cell depending on the stimulating cytokine and on the biochemical phenotype of the cell. It should be emphasized that the cells realize their suicidal choice under conditions of expression of the intact alternative thanatogenous apparatus.

**3.2. Receptors and ligands.** Model experiments with excitotoxins, such as AMPA ((S)-α-amino-3-hydroxy-5-methylisooxazole-4-propionate), NMDA (N-methyl-D-aspartate), and cainate have shown that the key role in the option of the cell death in the case of neurons belongs to excitation of various subtypes of glutamate and non-glutamate receptors [53] (Table 3).

And the intensity of the necrotic destruction is easily regulated [51, 54, 108] not only by their antagonists, such as CND (6-cyano-7-nitroquinoxaline-2,3-dione) and LY293558 [103] but also in the underlying pathways of the stress signal transmission. The necrotic sensitivity

**Table 3.** Receptor apparatus involved in the induction and modulation of necrosis

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| Glutamate | hippocampal neurons HT22, cortical neurons | U0126 (–) | | [97] |
| NMDA | cerebellum neurons | N-acetylcarnosine (N-ac) (+) | N-ac (+) | [98] |
| NMDA | hippocampal neurons | calpain inhibitor I (–) | | [99] |
| Cainate, NMDA | cortical neurons | {BSO + GM1} (+)<br>{$Fe^{2+}$ + GM1} (+) | | [100] |
| Cainate | hippocampal neurons | removal of trophic factors (–), z-VAD.fmk (+) | | [101] |
| Insulin | cerebellum neurons | tyrosine kinase inhibitor, protein kinase C inhibitor (–), NMDA antagonist (–), cycloheximide (–) | | [54] |
| Removal of serum | C6 | DEX (+), RU28362 (+), RU38486 (–) | | [102] |
| Removal of glucose and oxygen | oligodendrocytes O-2A | removal of $Ca^{2+}$ (–), CND (–), LY293558 (–) | | [103] |
| Removal of glucose and oxygen | cerebellum neurons | mGluRI antagonist (–), mGluRI agonist (+) | | [51] |
| $Zn^{2+}$ | cortical neurons | insulin (+), cainate (+), BDNF (+), AMPA (+) | | [104]<br>[105] |
| Anti-CD95 | Jurkat T* | sodium nitroprusside (SNP) (+) | SNP (–) | [80] |
| Anti-CD95, TNF, TRAIL | T-lymphocytes* | *FADD* $^{(-/-)}$ (–), *RIP* $^{(-/-)}$ (–) | | [106] |
| Anti-CD95 | JB6 (Casp8 $^{(-/-)}$) | pyrrolidine dithiocarbamate (–) | | [107] |

of hippocampal cells to hypoxia and excitotoxins was suppressed by an inhibitor of ERK kinases, U0126 [97], or by inhibitors of calpain-type proteases [99]. An activation of oxidative processes with $Fe^{2+}$ or with buthionine-sulfoximine (BSO) in the presence of monosialoganglioside (GM1) intensified the death of cortical neurons [100].

Under certain conditions, such survival factors as insulin (exposure for 48 h, culture of mouse cortical neurons) and the neuron growth factor (NGF) (absence of glucose, the HT22 culture of hippocampal neurons) initiated the necrotic program, but it could be suppressed by inhibitors of protein synthesis, of tyrosine kinases, and of protein kinase C [54, 109]. It is suggested that such a paradoxical role of neurotrophins in the absence or in the case of inhibition of TrkA-receptors (NGF-receptor family) should be due to binding of these survival factors to p75(NTR) from the TNF-receptor superfamily and to induction of lethal signals [110]. Insulin and neurotrophic brain factor (BDNF) promoted necrotic lysis of cortical neurons induced by $Zn^{2+}$, which was limited only by antioxidants [104, 105]. However, a known antioxidant N-acetylcarnosine increased both apoptosis and necrosis of neurons caused by NMDA [98].

Under varied conditions, the TNF-receptor family can initiate not only apoptotic but also necrotic cell death [111]. A necrotic lysis of L929 cells under the influence of TNF was determined by the lethal domain TNFR-55 [112]. Ligation of CD95 caused a caspase-independent necrotic death of activated T-lymphocytes that was abolished in cells dominant-negative in *RIP* $^{(-/-)}$ or *FADD* $^{(-/-)}$ genes. The necrotic effect of TRAIL, a ligand of the lethal receptors DR4 and DR5, also depended on the presence of a receptor-bound protein RIP [106]. Thus, under certain conditions (the absence or inhibition of caspases [107], the generation of $NO^{\cdot}$ [80]), the previously well-known apoptogenic receptors, in particular, CD95 can initiate the necrotic program.

Receptor-dependent pathways limiting necrosis have also been described. The death of neurons induced by ischemia/reperfusion (I/R) or by cainate was significantly intensified in mice with "knocked-out" p55 and p75 genes of the TNF-receptors. This suggests a protective function of a cytokine which in response to necrotic stress acts on the corresponding receptors as an autocrine agent [113]. This protection can be also contributed by a protein A20 (a "zinc finger" protein) whose expression is induced by TNF. An increase in the resistance to TNF of L929 cells overexpressing A20 was accompanied by a suppression of activities of phospholipases A(2), C, and D, and also of ROS generation by mitochondria [114]. Similar data on the protective effect of TNF have also been obtained with an infective stimulus [44].

Growth factors TGFα and EGRF also displayed an autocrine protective effect. An addition of antibodies neutralizing these growth factors into the DU145 culture of irradiated prostate carcinoma cells increased the fraction of necrotic cells [115]. Glucocorticoid receptors determined the survival mechanism of sympathetic neurons. The cell treatment with dexamethasone (DEX) prevented a necrotic lysis caused by co-culture with macrophages [43]. On the contrary, the necrotic death of glioma cells (C6) caused by serum removal was promoted by DEX or by RU28362, an agonist of glucocorticoid receptors, and was abolished by their antagonist RU38486 [102].

The activation of purinergic receptors by exogenous ATP seems to be rather a common induction mechanism of the necrotic death of target cells. In mesangial cells ATP induced both apoptosis and the receptor-associated generation of pores in the plasma membrane that is specific for necrosis [116]. *Pseudomonas aeruginosa s*imilarly caused the necrotic death of macrophages in culture through the activation of purine receptors [117]. Unlike apoptotic death, necrotic death of myeloid cells in the presence of ATP was caspase-independent [118].

Thus, under certain conditions, different receptors in cells, including those responsible for transmission of the apoptotic signal, initiate necrotic programs of cell destruction. However, there are also mechanisms and receptors not only for realization of one or another form of the death but also for the auto- or paracrine protection of the cells against conversion of a stress signal to the lethal one.

**3.3. Lipids.** An early appearance in cells of ceramide (CR) which is a hydrolysis product of a cell membrane lipid sphingomyelin is a response to very different stress stimuli: CD95/CD95, TNF, serum removal, genotoxic agents [79, 119]. The use of penetrating analogs of CR (N-acetylsphingosine or C2) as an inducer of the cell death makes it possible to differentiate signal pathways resulting in the execution of the lethal program (Table 4). Necrotic sensitivity to CR is displayed by a variety of cells: mouse macrophages and tumor cells, human T- and B-cells, rat hepatocytes. It is suggested that in the cell lines (e.g., Jurkat) where caspases are CR-activated, apoptotic mechanisms should be switched on, but on the involvement of $NO^{\cdot}$ they can be switched to the execution of necrosis [95]. Obviously, the transmission of the ceramide signal also includes redox-sensitive elements of the thanatogenous apparatus that is displayed by inhibition of NAC necrosis [120]. Incomprehensibly, the T-lymphocyte stimulation with phytohemagglutinin switched the CR-induced necrotic program to the apoptotic one [122]. Sphingosine, which is another product of sphingomyelin hydrolysis, also had a thanatogenous activity. At concentrations higher than 10 μM, this lipid induced necrosis in the human kidney cell culture HK-2, but at lower concentrations it promoted the cell resistance to exhaustion of ATP in the presence of $Ca^{2+}$-ionophores [123].

It is interesting that quasi-apoptotic morphological phenomena can be displayed in the absence of ATP. Thus, the generation of CR by acid sphingomyelinase results in the appearance of apoptotic distention-like vesicular structures in the plasma membrane [131, 132]. The so-called mitotic death is also suggested to be associated with the toxicity of CR which is gradually accumulated during the proliferation of damaged cells [119].

Products of lipid peroxidation (LPO) and lipids themselves play an important role in the translation of thanatogenous signals. The cellular necrosis induced by them can be suppressed at different stages of the lethal program realization by inhibition of the lipid incorporation into the cell (brefeldin A and lovastatin) [125], by binding of $Ca^{2+}$ [127], or by maintaining of the cell redox status by glutathione precursors [126]. Oxidized sterols were also toxic. In the human fibroblast MRCS culture, these compounds caused necrotic cell death, whereas in endothelial and smooth muscle cells they induced apoptotic destruction [129]. The bioactive product of LPO 4-hydroxynonenal (HNE) stimulated necrotic death in the culture of neurons, and this death could be prevented by chelating of the cytoplasmic $Ca^{2+}$ (BAPTA), by inhibition of the mitochondrial absorption of $Ca^{2+}$ with ruthenium red (RR), and by the MMP blocker cyclosporin A (CsA) [128].

A cholesterol metabolite bile glycochenodeoxycholic acid (GCDC) induced in hepatocytes a necrotic destruction that was promoted with the SH-reagent 1-bromohexane and suppressed with N-acetylcysteine (NAC) and with GC-EE [130].

Thus, along with other physiological mediators, natural lipids are inducers of the necrotic program in cells, and some stages of this program can be targets for various inhibitors of necrosis.

**3.4. Ionic channels.** Calcium is one of the most powerful inducers and mediators of cell death [16, 133-135] (Table 5). The cell choice of one or another death pathway under the influence of this ion that is usually modeled by $Ca^{2+}$-ionophores (A23187, Br-A23187) depends on

**Table 4.** Modulation of necrosis induced by natural and synthetic lipids

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| C2-CR + IFN-γ | C3H/OuJ | NMMA (±) | | [95] |
| C6-CR + SNP | Jurkat T* | SNP (+) | | [80] |
| C2-CR | JB6 (casp8$^{(-/-)}$) | NAC (–) | NAC (±) | [120] |
| Ceramides | hepatocytes | | | [121] |
| C2-CR | T-lymphocytes*, B-CLL leukemia cells*, B-lymphocytes* | PHA (–) | PHA (+) | [122] [124] |
| ox-LDL | JMN-18; Caco-2; HT-1080; AG-01519 | lovastatin (–), brefeldin A (–) | | [125] |
| ox-LDL | mesangial cells | α-tocopherol (–), probucol (–), NAC (–), GC-EE (–) | | [126] |
| ox-LDL | lymphocytes*, HL-60* (BCL-2$^{(+/+)}$) | EGTA (–) A23187 (+) | | [127] |
| HNE | hippocampal neurons | BAPTA (–), RR (–), CsA (–) | BAPTA (–), RR (–), CsA (–) | [128] |
| 7-βHC, 7-βKC | fibroblasts* | Ac-DEVD.cho (±) | | [129] |
| GCDC | hepatocytes | 1-bromoheptane (+), NAC (–), GC-EE (–) | 1-bromoheptane (±), NAC (–), GC-EE (–) | [130] |

Note: GC-EE, ethyl ether of γ-glutamyl cysteine.

the cell type or on the microenvironment [136-139] and can be determined by expression of the Bcl-2 family intracellular inhibitors of apoptosis [140].

The removal of $Ca^{2+}$ from the medium or an addition of chelators (EGTA, BAPTA) protected the cells against necrosis induced by starvation and anoxia [103], ox-LDL [127]. But this does not suggest the passive diffusion of the ion through membranes. Both the extracellular $Ca^{2+}$ flowing inside and its elimination from the intracellular depots are active processes depending on the functions of specific channels. Their inhibition with antagonists (nifedipine, benidipine, methoxyverapamil-D600, $Ni^{2+}$, $Cd^{2+}$) significantly limited the cell destruction induced by starvation [109], TNFα, cycloheximide [114], and rotavirus [34]. The realization of the suicidal program stimulated by cell overloading with $Ca^{2+}$ depends not only on functions of ion channels, but also on other factors [143]. On the necrosis of neurons and astroglia induced by an activator of ion- and receptor-dependent $Ca^{2+}$-channels, the inhibitor of calpain II maitotoxin suppressed both the specific processing of α-spectrin and lysis, whereas caspases were inhibited less effectively [142]. Inhibitors of mitochondrial absorption of $Ca^{2+}$ (ruthenium red, BAPTA) inhibited the ionophore-dependent necrosis of neurons similarly to cyclosporin A [128].

But in a culture of cerebellum neurons the toxic effect of cainate was strengthened by agents preventing an increase in the intracellular content of $Ca^{2+}$: by nifedipine and $Cd^{2+}$ through the plasma membrane but not by ryanodine (RND) and thapsigargin (TSG) from the endoplasmic reticulum. Antagonists of AMPA and of ryanodine receptors, such as LY303070 and caffeine, suppressed the necrosis induced by excitotoxin [108].

Univalent ions can contribute to the determination of cell lethal response to stress. The necrotic effect of veratridine (an activator of $Na^+$-channels) on sympathetic neurons was suppressed in the medium with a low content of the ion, an excess of $K^+$, and the inhibitor of the $Na^+/H^+$-exchange 5-(N-ethyl-N-isopropyl)amiloride (EIA) [145]. The death of monocytes of the THP-1 line induced by $K^+$ with the ionophore nigericin was decelerated by $Na^+$ and by inhibition of caspases [144].

Thus, the ion environment, the functional state of ion channels, and the ability of cells to maintain (or to endure) a certain content of intracellular ions can influence the choice of the suicidal elimination program [133].

**Table 5.** Effects on necrosis of agonists and antagonists of ion channels

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| Cycloheximide, TNF-α | mouse mesangial cells | nifedipine (–), benidipine (–) | nifedipine (–), benidipine (–) | [141] |
| Removal of glucose + NGF | mouse pheochromocytoma (PC12) | nifedipine (–), cycloheximide (–) | NGF (±) | [109] |
| Cainate | cerebellum neurons | LY 303070 (–), caffeine (–), $Cd^{2+}$ (+), nifedipine (+), TSG (+), RND (+) | nifedipine (±), z-VAD.cho (±) | [108] |
| Maitotoxin | PC12 | inhibitor of calpain II (–) | | [142] |
| $CaCl_2$ | neuroblastoma* (SH-SY5Y) | $Ni^{2+}$ (–) | | [143] |
| Nigericin | monocytes* (THP-1) | $K^+$(+), $Na^+$(–) | | [144] |
| $Na^+$ + veratridine | sympathetic neurons | $K^+$ (–), EIA (–), BAPTA (±) | | [145] |
| Rotavirus | enterocytes* (MA104) | $Ca^{2+}$ (+), BAPTA (–), D600 (–), TSG (+) | | [34] |

**3.5. Redox signal pathways.** The generation in the body of reactive oxygen species (ROS) and of reactive nitrogen species (RNS) can be increased by immunological reactions, by dysfunction of mitochondria during the realization of lethal programs, and by signal transmission. The importance of the last mechanism is suggested by the finding that the dominant lethal mutation Rac-1 by the minor GTP-protein suppressed the production of ROS and of lipid peroxides, the activation of NF-κB, and the I/R-induced necrosis of hepatocytes [146]. Because of high toxicity of oxygen, aerobic cells have multiple systems of antioxidative protection [147], and in some cases hypoxia prevents the realization of necrotic death, e.g., caused by a vitamin A derivative [79] or by TNFα [81] (Table 6).

The ROS component hydrogen peroxide is intensively produced in cells both as a factor of the immune defense and under conditions of various cell stresses; therefore, it is used in models of pathophysiological exposures associated with the activation of redox-dependent mechanisms. Hydrogen peroxide induces both apoptotic and necrotic cell destruction [148]. SH-containing agents (glutathione (GSH), NAC, etc.) also protect against oxidants, $H_2O_2$, and $O_2^{\cdot-}$ [71, 149]. The same effect NAC was displayed in a phagocyte-target model preventing necrosis of neuroblastoma cells cultured with microglia [42].

Unexpected features of $H_2O_2$ were found when it was used together with pharmaceuticals. In experiments with antitumor drugs, subtoxic doses of $H_2O_2$ switched the suicidal cell destruction to necrosis. It seems to be associated with signal functions of $H_2O_2$ because the inhibition of caspase activities or the activation of PARP requires significantly higher doses of hydrogen peroxide [154, 160].

ROS start playing a disastrous role together with other changes in the cell redox homeostasis: decrease in the contents of glutathione and thioredoxin and expenditure of NADPH [27]. A $Cd^{2+}$-induced apoptosis in U-937 cells changed to necrosis on the inhibition of glutathione synthesis [161]. An oxidative necrosis of neurons in the presence of BSO was increased with monosialoganglioside [100]. On the removal of GSH with 1-bromoheptane, a bile glycochenodeoxycholic acid also stimulated necrosis in hepatocytes that could be prevented by γ-glutamyl cysteine ethyl ester (GC-EE) [130]. However, the cell sensitivity to the necrotic destruction on the removal of GSH can also depend on other factors, e.g., on *Bcl-2* expression [162]. Both apoptosis and necrosis induced by the SH-reagent iodoacetamide were suppressed by an antioxidant N,N′-diphenyl-*p*-phenylenediamine (DPPD) [152]. NAC inhibited thermonecrosis and necrotic cell destruction of neuroblastoma by glial cells [42, 155]. Thus, the maintaining of the GSH level in cells or, more exactly, of a certain level of radical scavengers, in some cases prevents cell necrosis [126, 139, 153, 154, 163].

Interesting results have been obtained in the case of menadione-initiated cell death. The change in the NIH3T3 cell phenotype by transfection with SV40-T-

**Table 6.** Role of oxidants, antioxidants, and of nitric oxide in necrotic cell death

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| $H_2O_2$ | 3DO | NAC (–) | NAC (+) | [150] |
| $H_2O_2$ | lymphocytes* | vitamin C (+) | vitamin C (–) | [151] |
| Iodoacetamide | kidney epitheliocytes LLC-PK1 | DPPD (–), dithiothreitol (–) | DPPD (+), dithiothreitol (–) | [152] |
| VP-16, Ara-C, doxorubicin, *cis*-platinum, A23187, etoposide | Burkitt's lymphoma | $H_2O_2$ (+), dimethyl sulfoxide (–), tempol (–), desferol (–) | $H_2O_2$ (–), 3-AB (–) | [153, 154] [139] |
| 48°C | osteoblasts | NAC (–) | NAC (±) | [155] |
| Removal of NGF, β-amyloid | PC12 | SNAP (–), PPF (–), 8-bromo-GMP (–) | PPF (–) | [156] |
| Retinoic acid | SK-N-BE (2) | SNP (+) | SNP (–) | [80] |
| Anti-CD95 | Jurkat T* | nitrosoglutathione (+) | nitrosoglutathione (–) | [157] |
| SNP | RAW264.7 | $FeSO_4$ (+) | $FeSO_4$ (–) | [158] |
| Etoposide, *cis*-platinum | rat mesangial cells | L-arginine (–) | L-arginine (–) | [159] |

antigen promoted the necrotic death of fibroblasts. Although caspase-3 and caspase-8 were not activated, the necrosis was suppressed by inhibition of the CD95-receptor and also with NAC [164]. The effect of menadione on the lymphosarcoma P388 cells carrying the mutant p53 gene was studied by electron microscopy, and the choice between apoptosis and necrosis was found to depend on the menadione concentration and on the exposure time [165]. Low concentration of the drug induced a p53-independent apoptosis. The higher concentrations of menadione and a short-term exposure resulted in both morphological types of cell death. A prolonged influence of the prooxidant caused the necrotic cell death associated with early disorders in mitochondrial structure.

$NO^{\bullet}$ is now intensively studied as a mediator of various pathophysiological processes accompanied by ejection of cytokines and by cell death, and $NO^{\bullet}$ both protects against death [71] and promotes cell destruction [62, 89, 95, 166] (Table 6). Note, that the nitrosylation/denitrosylation reaction is involved in the activation mechanism of caspase-3 zymogen, which is an active contributor to apoptotic programs [167]. It seems that at a sufficient content of $NO^{\bullet}$, the equilibrium can be displaced towards the nitrosylated inactive zymogen and, thus, inhibit the apoptotic mechanisms.

One of the schemes of the thanatogenous effect of $NO^{\bullet}$ suggests its binding to the iron of various hemes, especially of the mitochondrial respiration chain, and their inactivation [157, 158, 168, 169]. A catalytic nitrosylation of thiol groups, in particular, in caspases and transglutaminase, can be a mechanism of the switching the apoptotic cell destruction to necrosis under the influence of staurosporine, CR, CD95-ligand, and retinoid, along with the suppression of apoptosis [80, 170, 171]. A damaging effect of exogenous sources of $NO^{\bullet}$ was suppressed by NAC or by donors of SH-groups and was increased by $Fe^{2+}$ or by inhibition of the GSH synthesis [158, 172-175].

The significance of $NO^{\bullet}$ in cell survival is supported by data on the induction of necrosis in cardiomyocytes by a prolonged diet including an inhibitor of NOS [175]. An addition into the culture medium of the i-NOS substrate L-arginine protected mesangial rat cells against the toxic effects of etoposide and platinum [159]. The $NO^{\bullet}$ generator S-nitroso-N-penicillamine (SNAP) suppressed the necrosis in the pheochromocytoma P12 cells by stimulation of an increase in their content of cGMP, similarly to the action of the phosphodiesterase inhibitor propentofellin (PPF) [156].

Peroxynitrite is a highly active nitrogen compound produced in the body. The maintaining of the glutathione level with its precursor NAC in the peroxynitrite-treated neurons [174] or in the NT2N cells [172] prevented the induction of necrosis.

In most cases, antioxidants suppress both the necrotic and apoptotic programs of the cell death [130, 152]. Nevertheless, there are also other examples: NAC in the 3DO cells suppressed necrosis and stimulated the $H_2O_2$-induced apoptosis [150], whereas vitamin C, unlikely, inhibited apoptosis in human lymphocytes [151].

Thus, an oxidative/nitrosotative stress induces necrosis in the cases when the intracellular reducing content is exhausted in the cases of excess generation of ROS and RNS, of the damage of systems of natural antioxidant protection, and of the insufficiency of ATP level.

**3.6. Poly(ADP-riboso)-polymerase.** Poly(ADP-riboso)-polymerase (PARP) is a nuclear enzyme containing a Zn-binding sequence. It is activated by DNA-breaks and joins 50-200-link oligomers of ADP-ribose to various proteins and to itself.

It is suggested that an excess activation of PARP, in particular, due to a mass-scale induction of DNA-breaks should be a cause of cell death because it results in a dramatic fall of the ATP level associated with its expenditure for the synthesis of $NAD^+$ which is a substrate of the enzyme [176]. An induction of DNA-breaks with hydroperoxide results in an extreme activation of PARP and in a subsequent exhaustion of $NAD^+$ and ATP. An inhibitor of PARP (1,5-dihydroxyisoquinoline, 3-aminobenzamide (3-AB), nicotinamide) significantly postponed cell lysis [154, 177] or switched it to the apoptotic pathway [160, 178], alongside with a significant increase in the activities of caspases [179]. In the case of inefficiency of PARP activation mechanisms, as it is in cells with a mutant enzyme containing a caspase-resistant sequence in the place of cut, the cells became more sensitive to necrosis induced by UV radiation [182] or by TNF-α. In the latter model of fibroblasts with the caspase-resistant PARP, inhibition of this enzyme also suppressed necrosis and increased apoptosis, similarly to the parental cells [181]. An inhibition of PARP suppressed both necrosis and apoptosis in the case of infection of HeLa and P1 cells with parvovirus [35]. Thus, a common pathway for prevention of the necrotic elimination of cells is the proteolytic or pharmacological inactivation of PARP (Table 7).

Nevertheless, PARP is fragmented and, as result, inactivated also in cells during their necrosis, although the fragments are unlike those generated during apoptosis [183]. This is probably due to specific features of the proteolytic apparatus responsible for execution of the destruction program, but it also can be due to specific functions of the resulting peptides, and these functions are associated with the involvement of PARP not only in maintaining of functions of the reparation apparatus of DNA [184, 176] but also with the stimulation of the apoptotic endonucleolysis. Therefore, the switching of necrosis to the apoptotic pathway with 3-AB can be free of the ATP retention by the inhibition of PARP [160, 185]. Thus, a "knock-out" of the corresponding gene had no effect on the intensity of apoptosis of thymocytes [180]. A suppression of necrosis which had been induced with subtoxic doses of $H_2O_2$ combined with antitumor drugs by inhibition of PARP is unlikely to be due to its superactivation [154, 160]. Data on the induction of necrosis by irradiation of HL-60 cells at the dose of 10 Gy and of apoptosis at the dose of 50 Gy also fail to support the concept of ATP exhaustion because of the PARP activation [186].

Findings of new members of the PARP family suggest a more complicated (not only through ATP exhaustion) mechanism of this enzyme involvement in the modulation of the cell resistance to pathophysiological exposures and in the programs of suicidal destruction. An involvement of PARP into the regulation of activity of the transcriptional redox-stimulated factor NFκB seems to be important for the survival program executed by the receptor complex of TRADD/RIP/TRAF2 and by this factor [187].

**Table 7.** Inhibitors of poly(ADP-riboso)-polymerase (PARP) as switches of necrosis to apoptosis

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| $H_2O_2$ | promonocytes* (U937), LLC-PK1 | 3-AB (–) | 3-AB (+) | [160] [177] |
| $H_2O_2$, streptosotocin | β-cells*, rat β-cells | nicotinamide (–) | nicotinamide (+) | [178] |
| Peroxynitrite | thymocytes | 3-AB (–), *Parp*$^{(-/-)}$ (–) | 3-AB (+), *Parp*$^{(-/-)}$ (±) | [180] |
| Parvovirus H-1 | HeLa, P1 | (i) PARP (–) | (i) PARP (–) | [35] |
| TNF-α | fibroblasts | 3-AB (–) | 3-AB (+) | [181] |

Note: Fibroblasts are cells with the caspase-resistant PARP.

**3.7. Proteases.** In the apparatus of the suicidal elimination of cells, proteolytic enzymes are responsible for several functions, including activation of receptors, the transmission of the lethal signal, and the execution of destruction of various biopolymers. Cysteine proteases of the caspase family play an essential role in these processes. Therefore, in the absence of caspase expression or in the presence of endogenous or exogenous inhibitors, the destructive programs are switched to the necrotic pathway [15, 188]. Thus, human kidney carcinoma cells (LCC) deficient in caspase activation died in the presence of the zinc chelator TPEN via necrosis, whereas other cell lines displayed a caspase-dependent apoptosis [189]. Caspase inhibitors switched to necrosis the cell death caused by ionizing radiation [190], camptotecin [191], etoposide, dexamethasone, and inducers of MMP [192]. The apoptotic death of keratocytes was prevented by the pancaspase inhibitor z-VAD.fmk, along with stimulation of necrotic death [193]. B-Lymphocytes were similarly affected by this inhibitor [194]. In the presence of caspase inhibitors, the PCD forms depending on purine receptors were also switched to necrosis [118]. Even after the release of cytochrome C from mitochondria, the inhibition of caspases 3/7 by exogenous NO also turned the death pathway to necrosis [170]. This seemed to be associated with the protease-inhibiting activity of non-heme nitrosyl complexes [158].

However, in the case of some other stress stimuli and cell lines, the necrotic program to be realized needs caspase activation [196, 197, 201] (Table 8). The death induced by removal of ATP in CD95-stimulated cells of the Jurkat line, along with the apoptotic program, was suppressed with the pancaspase inhibitor z-VAD.fmk [198]. In the presence of this caspase inhibitor but not in the presence of z-DEVD.fmk (an inhibitor of caspase-3-like proteases), the TNF-induced necrosis in enterocytes and fibrosarcoma cells was limited [93, 94]. The inhibition of caspases also suppressed the necrosis caused by toxin A of *C. difficile*, by toxin α of *S. aureus*, ouabain, and nigericin [146]. The involvement of caspases in necrosis is confirmed by splitting not only of PARP [38, 183] but also of other substrates; thus, caspase-6 seems to be involved in the fragmentation of lamine B induced by a necrotic dose of IR [186].

And, finally, another caspase-dependent model of necrosis has been studied on fibrosarcoma L929 cells. The inhibition of caspases in these experiments increased by three orders of magnitude the cell sensitivity to TNF-induced necrosis. A similar effect was observed at the excitotoxic death of the hippocampal neurons [101]. These findings have also shown an anti-necrotic role of caspases. In these cases they are suggested to be involved together with other proteases into the catabolic apparatus (apoptosome) that is responsible for elimination of "worthless" mitochondria producing excess amounts of ROS. On the failure of such an attempt, necrosis is initiated in the cell [90]. This "programmed" autophagy of mitochondria that occurs with the involvement of the same proteases (caspases) as the apoptotic destruction of cells is exactly described by the term "mitoptosis" [202].

**Table 8.** Inhibition and activation of necrosis by inhibitors of caspases

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| TNF-α | IEC-6 | z-VAD.fmk (–)<br>z-DEVD.fmk (±) | | [94] |
| Toxin A of *C. difficile*, toxin α of *S. aureus* | THP-1* | z-VAD.fmk (–) | | [37]<br>[144] |
| Hypoxia | protein kinases of rat kidneys | z-D.dcb (–), PD150606 (–) | | [195] |
| Removal of glucose + KCN/rotenone/antimycin | PC12, Hep G2 | Bcl-2 (–), Bcl-$X_L$ (–),<br>CrmA (–), Ac-YVAD.cho (–) | | [196, 197] |
| Anti-CD95 + OLM,<br>anti-CD95 + GSNO | Jurkat T* | z-VAD.fmk (–)<br>z-VAD.fmk (–) | z-VAD.fmk (–) | [198]<br>[157] |
| 4-HPR | kinases of neuroblastoma | hypoxia (–), boc-D.fmk (±) | hypoxia (–),<br>boc-D.fmk (–) | [79] |
| $Na_2AsO_3$ | U937 | z-VAD.fmk (+) | z-VAD.fmk (–) | [199] |
| $Zn^{2+}$ | MOLT-4 | Ac-DEVD.cho (±)<br>Ac-YVAD.cho (±) | Ac-DEVD.cho (±)<br>Ac-YVAD.cho (±) | [200] |

The number of reports on cell destruction mechanisms not needing caspases is increasing. In particular, $Zn^{2+}$ induced in MOLT-4 cells both forms of death, and they were suppressed neither with Ac-DEVD.fmk nor with Ac-YVAD.fmk (inhibitors of caspase-3- and caspase-1-like proteases) [200]. Serine proteases are involved in TNF-induced necrosis [90] and in the death caused by chemical hypoxia [38]. Other enzymes, including cysteine calpain-type proteases also seem to play the role of caspases [203]. An injection of the calpain protease inhibitor cbz-LLY.CHN2 essentially prevented apoptosis and necrosis in hepatocytes and nonparenchymal cells during the I/R of rat liver [204]. NMDA-induced necrotic death of neurons was switched to apoptotic death on inhibition of a $Ca^{2+}$-activated protease calpain I [99]. An inhibitor of calpain II inhibited the $Ca^{2+}$-induced death of neurons [142].

Unlike apoptotic death, necrotic death is associated with significantly decreased activity of ectoproteases acting on the external surface of the cells [205]. Data on increased necrotic sensitivity of cells transfected by mutant protein suggest that excitotoxic death should involve presenelin-1, a transmembrane protein with properties of the protease which processes β-amyloid and is expressed in Alzheimer's disease [206].

Thus, the role of caspases as the main "executor of apoptosis" in necrotic programs is more varied. Their inhibition can result in both suppression and amplification of necrosis. The suppression of necrosis seems to reflect the role of caspases in the activation of hydrolases specific for this program. The role of ATP in these mechanisms remains unclear. In some cases, the switching of apoptosis to necrosis in the presence of caspase inhibitors seems to be associated with energetics. If the inhibition of caspases prevents the inactivation of PARP and, correspondingly, stimulates the "self-eating" of ATP, this is a direct stimulus for realization of the energy-independent programs of necrotic death. And by contrast, the inactivation of PARP with caspases supports the energy-dependent apoptotic processes [182]. And, finally, there are caspase-independent programs executed with involvement of other proteases, such as calpain or serine proteases, which first of all induce damage in the plasma membrane and cell organelles [83].

**3.8. Mitochondria.** Mitochondria are involved in the realization of a stress program resulting in cell death by several pathways that can be combined. Similarly to a cellular power station, they determine the choice in favor of ATP-dependent or of ATP-independent lethal programs [207]. Being a source of thanatogenous activators, they initiate caspase-dependent mechanisms and directly control the executive stage of apoptosis, and in the extramitochondrial programs (CD95-like) they amplify the activation of proteolytic and nucleolytic hydrolases [208, 209]. Their thanatogenous functions are realized via membrane depolarization (decrease in $\Delta\Psi_m$), increase in the permeability of the external mitochondrial membrane pores (MMP), a decrease in ATP content, and via releasing caspase activators: cytochrome C, an activator of nucleases AIF, and of other proteins, such as arginase I, Hsp10, Hsp60 [85, 171, 210]. And, finally, they are sources of ROS and RNS which are also involved in the choice of the form of cell suicidal destruction.

Obviously, the retention of a certain level of the energy carrier ATP is a prerequisite for apoptotic programs [168, 211, 212]. And the ATP-dependent links of apoptosis can be both precede or follow the stages which are associated with activation of caspase-3-like proteases [211]. Inhibitors of respiration induce necrosis in many models. Rotenone, an inhibitor of complex I, stimulated cell necrosis in the absence of glucose [157]. The similar inhibitor 6-hydroxytryptamine used in experiments modeling cell diminution in Parkinson's disease also caused necrosis of the PC12 neuroblastoma. Note, that this death was not inhibited by antioxidants [213]. However, early stages of necrosis were significantly postponed by melatonin, a hormone with antioxidant features [214]. 3-Nitropropionic acid, an inhibitor of mitochondrial complex II, induced necrosis in primary culture of rat cortical neurons. An addition of astroglia cells or of a conditioned medium markedly limited the death of neurons [215]. On increasing the concentration in the medium of antimycin A, an inhibitor of complex III, apoptosis was shifted towards necrosis [84]. Many models of PARP-dependent apoptosis and necrosis are associated with a disastrous expenditure of ATP (section 3.6).

But if the glycolytic mechanism can maintain the level of ATP, an addition of glucose or of glutamine switches the death to apoptosis [157, 216]. Addition of oligomycin and fructose had the same effect on cells treated with a $Ca^{2+}$ ionophore, but concurrent addition of cyclosporin also prevented the apoptotic death of hepatocytes [138]. The last finding suggests that the prevention of the ATP generation and, let us emphasize, of the production of ROS, by mitochondria switches necrosis to the cytochrome C- or AIF-dependent death pathway characterized by a high-molecular-weight fragmentation of chromatin [210].

Disorders of a definite permeability of the membrane pores seem to be necessary for realization of lethal programs [217, 218]. Inducers of MMP and of apoptosis PRIX and mClCCP initiated necrosis in hepatocytes in the presence of the caspase inhibitor z-VAD.fmk [192]. The importance of the functional state of MMP is shown by data that both necrosis and apoptosis induced by different stimuli were effectively inhibited with pore blockers, such as cyclosporin A (CsA) and bongkrekic acid [87, 138, 171, 219].

It should be emphasized that in some cell lines even a 20-fold fall of the ATP content, caused, for example, by hypoxia and starvation, failed to significantly affect cell survival. But additional stress exposures, such as

hyperthermia, resulted in mass death of the cells [220, 221]. But, if the ATP generation is still maintained on the level sufficient for the activation of caspases in the cells executing the suicide program, there are additional mechanisms initiating necrotic death. Necrosis of AKR-2B fibroblasts induced by the removal of serum was not accompanied by a decrease in the ATP content and was suppressed by inhibition of caspases [222]. In fibrosarcoma L929 cells a necrotic program was realized under the influence of TNF at the retention of the ATP level and in the presence of intact apoptotic mechanisms [81]. This is also supported by data on the caspase-dependent pathways of realization of the necrotic program which are likely to need ATP or on the existence of ATP-independent mechanisms of caspase activation (section 3.7).

In addition to the ATP insufficiency, the role of signal for necrosis in some suicide programs can be played by by-products of mitochondrial respiration, in particular, by reactive oxygen species ($H_2O_2$, $O_2^{\cdot-}$) released at the first links of the mitochondrial chain of electron transport which are produced in both apoptotic and necrotic programs depending on activities of the pentose phosphate shunt and of its key enzyme transaldolase [223]. ROS cause a decrease in the amount of key cell reducers thioredoxin and glutathione. The most important role of glutathione is supported by the absence of mitochondria in the only glutathione-free eucaryote found [224]. The role of ROS is convincingly shown on the model of TNF-induced necrosis in L929 cells. Suicidal destruction of cells occurred at a sufficient level of ROS, but inhibition of mitochondrial complexes I or II suppressed this reaction [225]. And only the most lipophilic antioxidant, butylated hydroxyanisole, inhibited the cell death [81].

Thus, mitochondria are not only a target for various thanatogenous mediators, but also a source of three relatively independent lethal signals initiating apoptotic or necrotic outcome. These signals include a fall of the ATP content, ROS and RNS production (with the involvement of either cytoplasmic or mitochondrial NOS [226]), and the release of thanatogenous proteins. These signals are controlled depending on the expression of many factors including the proteins specific for various pathological syndromes: the FAC protein for Fanconi's anemia [227], the ATM for ataxia-telangiectasia [228], and the UCP1 and UCP2 for the fatty liver [229, 230].

**3.9. Products of protooncogenes and stress-regulated proteins.** Products of the *Bcl-2* gene and of its analogs are a large group of proteins which play the most important role in cell sensitivity to stress and lethal signals. Antiapoptotic proteins of the Bcl-2 family in many cases can inhibit both the apoptotic [201] and necrotic programs of cell destruction which are induced, for instance, by chemical anoxia [197, 198], β-amyloid [231], cytokines [62], $Ca^{2+}$-ionophore, a carcinogen and UV [136], and removal of $Mg^{2+}$ [232] (Table 9).

A complicated mechanism responsible for the balance between necrotic and apoptotic responses of cells reflects the interrelations between proapoptotic and anti-apoptotic proteins of the Bcl-2 family. The anti-necrotic effect of chronic hyperglycemia also includes the mechanism of Bcl-2 expression and inactivation via phosphorylation of the proapoptogenic protein Bad [233]. Excess expression of *Bax* stimulates the apoptotic program, but a coexpression of *Bcl-*$X_L$ switches it to the necrotic program [234]. It is important that the cell resistance to stress can be epigenetically determined by these proteins due to changes in the composition of oligomeric complexes or in the conformation under the influence of the microenvironment [235]. The role of catabolic and initiator proteases in the Bax-induced necrosis can be played by both cysteine [236] and serine proteases [237].

**Table 9.** Role of protooncogenes in induction and modulation of necrosis

| Stimulus | Cell line/tissue | Inhibitor (–)/activator (+) | | References |
|---|---|---|---|---|
| | | necrosis | apoptosis | |
| IR, 50 Gy | HL-60 | Bcl-2 (+) | Bcl-2 (–) | [186] |
| A23187, MNNG, UV | McA-RH8994 | Bcl-$X_L$ (–) | | [136] |
| β-amyloid + NGF (1 ng/ml) | PC12 | Bcl-$X_L$ (–) | Bcl-$X_L$ (–) | [231] |
| Removal of $Mg^{2+}$ | hybridoma cells | Bcl-2 (–) | | [232] |
| *cis*-Platinum | A-172<br>A-251 | Bcl-$X_L$ (+),<br>{Bcl-$X_L$ + Bax} (+) | Bax (+) | [234] |
| *BNIP*3 | HL-60 | cyclosporin A (–),<br>bongkrekic acid (–),<br>z-VAD (±) | | [219] |

As in the case of apoptosis, a gene has been detected that is responsible for necrotic death of the cells expressing it. Pronecrotic functions of a protein BNIP3 (an inducer of apoptosis of the Bcl-2 family which lacks a functional BH3 domain) in cells transfected with this gene were displayed by an early permeability of the plasma membrane, cytoplasm vacuolization, and autophagy of mitochondria. The morphological changes were accompanied by opening of pores in the mitochondrial membrane, a decrease in the $\Delta\Psi_m$, and an increase in ROS production. All these changes were inhibited by inhibitors of MMP: cyclosporin A and bongkrekic acid [219].

However, there are also destruction programs uncontrolled by the Bcl-2 family [238]. In particular, a necrotic effect of peroxynitrite [180] and of the respiration uncoupler 3-acetylpyridine [239] was not prevented by Bcl-2. Note, that both caspase-independent death pathways stimulated, in particular, by I/R and caspase-dependent pathways (apoptosis) are not always inhibited by an overexpression of Bcl-2 [26, 239]. One of the protective mechanisms includes the providing of ATP-dependent mechanisms of the cell survival and/or the inhibition of other catabolic enzymes, e.g., of serine proteases. In total, both the executing mechanisms of cell death (proapoptotic members of the Bcl-2 family) and the inhibiting ones (antiapoptotic members of the Bcl-2 family) have much in common for both apoptotic and necrotic cell destruction [198].

Apoptosis-inhibiting proteins (IAP) seem to be not involved in regulation of the necrosis program. Their expression in transfected cerebellum neurons had no effect on excitotoxic cell death [240].

The suicide response is also chosen with the involvement of other products of protooncogenes. Under exposure to IR, the expression of a mutant ATM-protein switched the death of the transfected cells to necrosis [230], and this was likely to be associated with suppression of the antiapoptotic system switched on by NF-κB [241].

Many data suggest that heat shock proteins (Hsp) should be involved in the lethal programs [155, 242]. The Hsp70 and Hsp27, well-known inhibitors of apoptosis induced by various stimuli, are studied the best of all [243, 244]. Moreover, their expression explains the cell prevention against necrosis induced by hyperthermia, ischemia, and oxidative stress [245]. In the transgenic mice overexpressing the Hsp70 the experimental ischemia/reperfusion caused a smaller area of the cardiac muscle damage, a lower level of blood creatinine (a necrosis indicator), and the mice displayed a faster recovery of contraction amplitude [246, 247]. The expression of the Hsp27 or of its homolog α-B-crystalline protected cardiomyocytes against ischemia-induced necrosis both *in vitro* and *in vivo* [248, 249].

The Hsp70-provided protection of myocardial cells against I/R was not associated with retention of ATP level during hypoxia; however, in heart tissue from Hsp70-expressing animals the ATP level after exposure was recovered faster than in the control [250]. These data suggest that chaperons should protect the mitochondrial functions during I/R and/or promote their recovery. A similar effect was observed in the case of overexpression of the mitochondrial chaperons Hsp60 and Hsp10 in cardiomyocytes. The prevention of cell death correlated with an increase in the activities of complexes III and IV and with better recovery of ATP [251]. Because neither Hsp70, nor Hsp27 are constituents of mitochondria, their anti-necrotic effect is unlikely to be associated with a direct effect on the structure of mitochondria. More likely, these chaperons suppress some necrotic pathways that results in deregulation of mitochondria and in cell death. These pathways include the p38 and JNK stress kinases. The activities of these MAP kinases increase during I/R, and the inhibition of their activities decreases necrotic sensitivity of cells [252, 253]. Because the activities of MAP kinases p38 and JNK are suppressed on Hsp70 expression [244], these kinases are suggested to be essential contributors to the anti-necrotic effect of Hsp70 in myocardium. The mechanism of the anti-necrotic activity of Hsp27/α-B-crystalline is yet unknown, but it also can be associated with the prevention of mitochondrial damage [249].

A protective effect of a preliminary moderate heating was mainly displayed during the expression of chaperons previously to initiation of the necrosis program by hydroperoxide [254], heating [255], I/R, and transplantation of myoblasts [256]. A preheating of HepG2 and Caco-2 cells before the treatment with an extract of the *Licania michauxii* (Rosaceae order) root also inhibited the necrotic effect of the latter. Nevertheless, the extract itself induced Hsp70 expression [257]. Necrotic death at 48°C in osteoblasts was also accompanied by Hsp70 expression [155]. In another model, notwithstanding Hsp27 and Hsp70 being induced only at a temperature below 42°C, heating inhibited the apoptotic reaction at 44°C and failed to affect the necrotic reaction at 46°C [255]. In some cases, the preliminary heating displayed specificity. Thus, a moderate heating prevented the necrotic death of smooth muscular cells of vessels induced by an acute heat shock but failed to prevent apoptosis caused by serum removal or by inhibition of protein kinases with staurosporine (STS) [258]. Deletion of the i-NOS gene resulted in protection of β-cells against the necrotic effect of cytokines and in concurrent suppression of activities of caspases and of Hsp70 expression [89]. The data presented suggest that Hsp proteins in the suicide destruction programs have two functions that are realized during different stages of the cell response to stress. Before the irreversible stage Hsp (similarly to other stress proteins) can act as protectors, whereas during the irreversible stage they seem to be secreted as markers and catalysts of necrosis [255, 259, 260]. Thus, an increase in the expression of glucose-regulated proteins (Grp78,

Grp94) but not in the Hsp expression prevented the lethal effect of iodoacetamide on kidney epithelium cells, whereas on the suppressed expression of these proteins the cell sensitivity was increased [152].

## 4. MECHANISMS OF NECROSIS AND ITS ROLE IN THE PROGRAMMED CELL RESPONSE TO STRESS

The data presented above clearly show the existence of several components of the thanatogenous apparatus of cells responsible for the realization of their necrotic destruction (Fig. 1). Depending on the type of exposure (stress) or of a pathophysiological stimulus (infection, radiation, xenobiotics, ischemia, etc.), the lethal program is executed by various links of this apparatus. The initiation of the necrotic program occurs with the involvement of such endogenous agents as TNF, TGF, neuromediators, neurotrophic factors, and ATP. Note that both the agents themselves and the receptors sensitive to them can be either expressed by cells constitutively or be caused by an autocrine or paracrine induction. The third target/sensor is DNA. It can be damaged either as a result of a direct action, e.g., of antitumor agents, or as a result of oxidative stress of ischemia/reperfusion. The activation of PARP that is inalienable of the damage of the DNA structure is usually thought to be associated with a disastrous expenditure of the polymerase substrate $NAD^+$ and, correspondingly, of ATP, and this is suggested to result in cell necrosis. However, the existence of necrotic programs not fitting this scheme [186] and also of programs not depending on PARP [157, 261] seems to suggest that PARP should play other roles in the mechanisms of survival and death [187].

Ion channels of both $Ca^{2+}$ and univalent ions are important receptors of the stress exposure resulting in necrotic elimination of "undesirable" cells. Through activation of cGMP, $NO^{\cdot}$ can play the determining role in their response to a stress "signal".

It becomes clear that ceramide is a common component of stress signals from a variety of sensors, e.g., from the TNF-receptor superfamily and DNA [119]. And without the involvement of ceramide in the transmission of a lethal signal, the necrotic program is not realized [95].

The inclusion of the MAP kinases ERK, JNK, and p38 seems to be a general stage for both the apoptotic and necrotic programs. In particular, both apoptosis and necrosis in rat pancreas β-cells was inhibited by combined use of inhibitors of the p38- and ERK1/2-MAPK-signal pathways. Inhibition of ERK reliably suppressed only the cytokine-induced apoptosis [88].

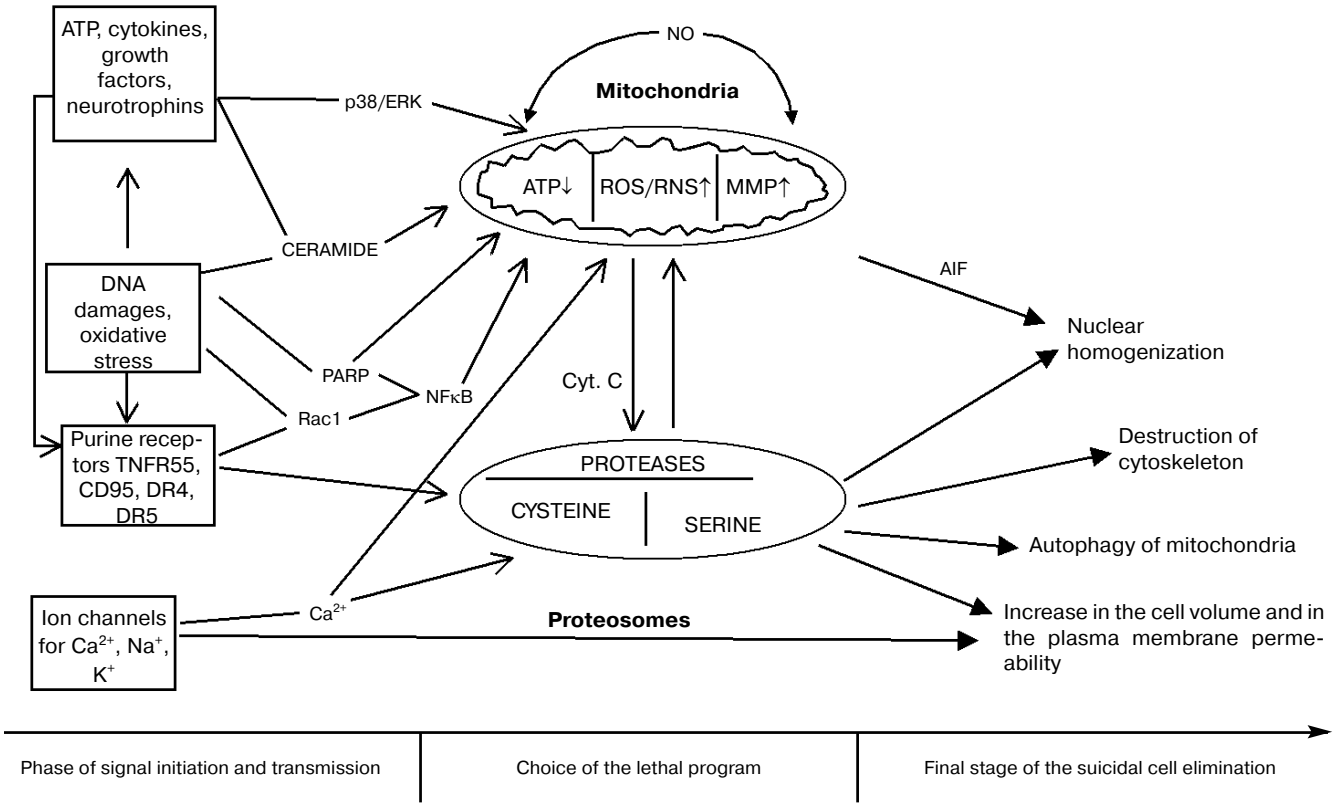


**Fig 1.** Possible pathways for realization of the necrotic form of programmed cell death.

An immense number of reports about the efficiency of antioxidants in the inhibition of cell necrosis suggests important roles redox-dependent signal pathways and of mitochondria as producers of ROS and RNS in the control of lethal programs. Moreover, mitochondria are a subject of the most important systems of the cell survival, in particular, of Bcl-2. The expression level of this family members [219], of their activities determined by the microenvironment [235], is a factor of stability, of resistance to a stress stimulus, and of the choice of the lethal program.

The activation of caspase-like hydrolases, calpains, and serine proteases is the last stage in suicidal cell destruction. Obviously, these proteases are also involved in the modulation of signal pathways and in the activities of mitochondria. But their role in the necrosis-like death programs, in the activation of nucleases and lipases, and in the destruction of the cytoskeleton and plasma membrane has only started to be elucidated.

Thus, the biochemical apparatus components and their regulation have much in common in the programs of elimination of "unwanted" cells executed with the retention of the plasma membrane integrity (apoptosis) and with its damage (necrosis). Virtually, this suggests that the necrosis phenomenon should be controlled in order to correct pathophysiological consequences associated with the realization of this program of cell death. Theoretically, this suggests that the necrosis program should be included into the general concept of PCD [6, 262] (Fig. 2). An essential element of this scheme should be the so-called "mitotic" death, or the death of mitotically active cells exposed to stress postponed by one or more divisions. This is supported by data on a significant correlation between the number of necroses and the parameters specific for the death of the dividing cells (a number of micronuclear cells, the survival) [115, 230, 263]. And the accumulation of ceramide during cell divisions seems to be a signal that triggers the suicidal destruction of aberrant cells via necrosis and thus prevents their expansion in the body [119].

Thus, a stress stimulus (not only a pathophysiological one, but also a stimulus that normally induces necrosis) initiates adaptive programs that in a reversible phase can result in the cell transition to rest, proliferation, or differentiation).

## 5. CONCLUSION

The elimination mechanism of an "undesirable" cell has also a fundamental significance for both the cell

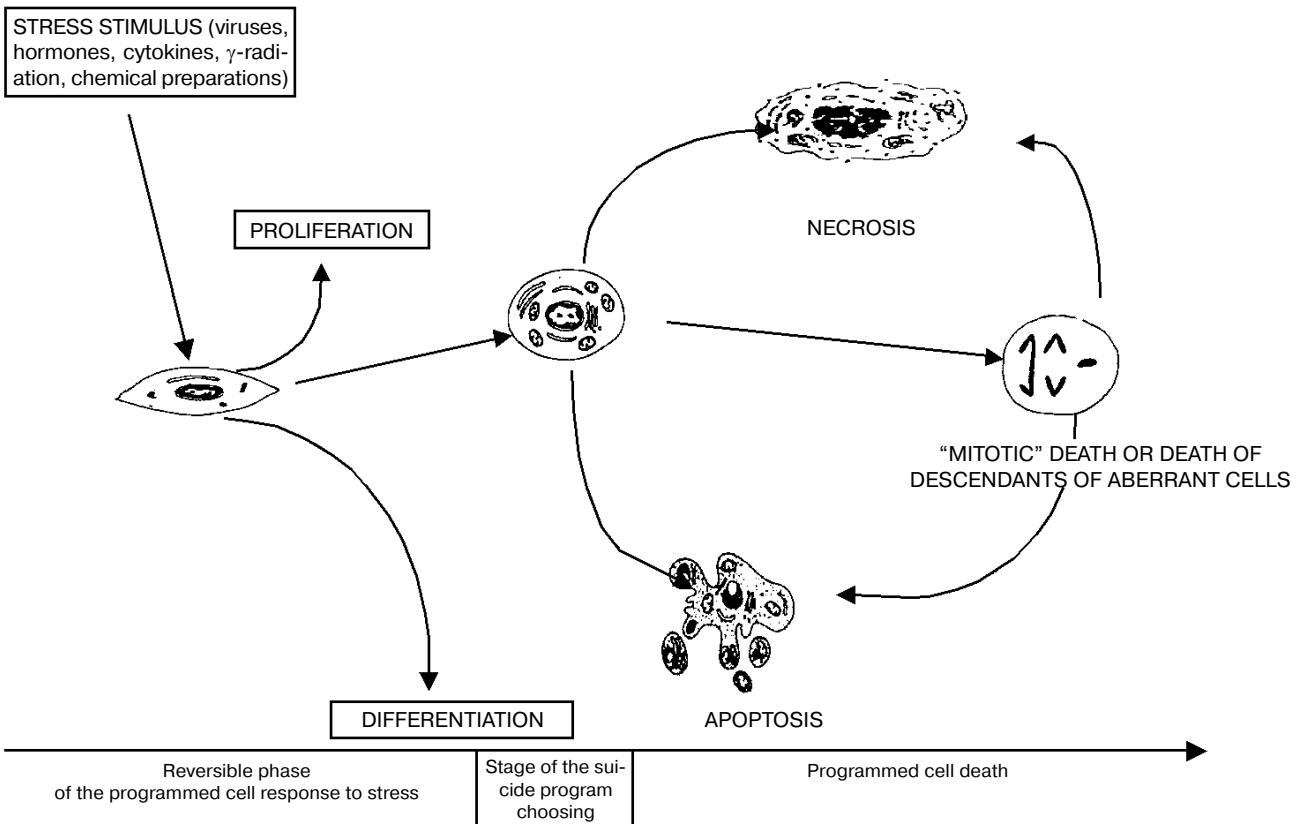


**Fig. 2.** Cell death and its place in the programmed cell response to stress.

renewal and pathophysiological consequences. A dying cell can be destroyed either through the apoptotically regulated phagocytosis [20, 264] or otherwise providing the prevention of the inflammatory burst [26, 82], or as a result of necrotic secretion of intracellular contents stimulating the inflammatory reaction of the environment [31, 71, 262, 265, 266].

This response includes various environmental cells depending on the initiating protein signals released from the dying cell. These protein signals include calreticulin, hsp90, hsp70, hsp10, etc. On the entrance into the extracellular space, these proteins stimulate an activation of antigen-presenting cells (APC), including dendritic cells [267]. The comparison of apoptotic and necrotic cell abilities to stimulate APC has shown that macrophages with these cells absorbed activated the T-response more effectively. And the apoptotic cells initiated in the APC a secretion of cytokines which inhibited the T-response [268].

During evolution, the alternative program of cell elimination could be secured by the requirement in the case of dangerous physiological situations (infection, trauma) for a stimulus provided just by necrosis for a rapid mobilization of the body protection: dendritic cells, monocytes, and neutrophils [81, 269-272]. Certainly, such response can change to a stage which is characterized by a self-maintaining inflammatory chain process. And the key role of mitochondria in cell necrosis is manifested by a close connection between the mitochondrial dysfunction and inflammatory response (induction of cytokines, activation of cyclooxygenases, synthesis of ROS) found in the pathogenesis of various diseases [48, 59, 273-276].

The above-presented data show an active and programmed character of necrotic death in the body and allow us to outline the prospects for control of this process in order to develop new strategies of the treatment, in particular, to elaborate necroprotectors, i.e., pharmacological agents to inhibit cell necrosis. And thanatogenous receptors, signal transmission mechanisms, $NO^{\bullet}$, PARP, catabolic hydrolases (especially serine and calpain proteases), and mitochondria can be important targets for a differentiated inhibition or activation of one or the other form of cell destruction [64].

## DEDUCTIONS

1. Necrotic death characterized by disturbance in the plasma membrane integrity and the effluence of the cell contents similarly to apoptosis is a form of programmed cell death.

2. The necrotic program can be realized in cells in the presence of an intact mechanisms of apoptotic cell destruction.

3. In many cases, the epigenetic mechanism of the necrotic cell death is mainly caused by disorganization of mitochondria and the corresponding insufficiency of ATP required for functioning of the energy-dependent transcription and translation processes.

4. The necrotic elimination of "undesirable" cells is suggested to be "the last frontier" in the expansion of infected or aberrant cells in the body.

5. A delicate balance between apoptosis and necrosis of cells in various tissues of the body is a balance between health and disease. A disturbance in this balance can cause development of aging-specific neurodegenerative and oncological diseases and also of pathophysiological processes initiated by ischemia and/or inflammation.

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