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REVIEW

Immunology of Apoptosis and Necrosis

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

[1]Medical Radiological Research Center, Russian Academy of Medical Sciences, ul. Koroleva 4, 249036 Obninsk, Russia; fax: (7-095) 965-1440; E-mail: pros@mrrc.obninsk.ru
[2]Department of Biochemistry, K-323, Boston University School of Medicine, 715 Albany St., Boston MA 02118, USA; fax: 617-638-5339

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Abstract—A complex of reactions regulating the number of cells in organs and tissues under normal and pathologic conditions is one of the most important systems of multicellular organisms. In this system, which controls both cell proliferation and clearance, clearance has been given special attention during the last three decades. Some stages of the clearance are known (the choice of "unwanted" cells, their destruction not affecting the surrounding tissue, and, finally, removal of the corpses), and undeniable progress has been achieved in the understanding of the second stage mechanisms, whereas mechanisms of elimination per se of cells or their fragments still continue to be *terra incognita*. The clearance of such cells is mainly determined by different components of natural and adaptive immunity: phagocytes, complement, opsonins, antigen-presenting cells, etc. Recently specific "danger signals", such as hydrolases, DNA, heat shock proteins, and other potential immunogens released by cells during their elimination have been discovered. Entering the extracellular space, these signals induce inflammation and injury of the surrounding tissues, i.e., autoimmune reactions. Heat shock proteins, in addition to chaperon activity, act as signaling, costimulating, and antigen-carrying molecules in the interactions of dying cells and the immune system.

Key words: programmed clearance of apoptotic and necrotic cells, immune system, danger signals, heat shock proteins

The definite size of organs and tissues in the living body is maintained by control of proliferation and removal of cells. Among stages of this control (the choice of cells to be eliminated, their destruction safe for the surrounding tissue, and removal of the corpses) mechanisms of cell destruction, especially apoptosis, have been given the greatest attention of researchers during the last three decades. But specific features of the elimination per se of cells or their components are still *terra incognita*.

The history of the system of cell elimination from an organism began, so to say, "from the tail". In 1882, I. I. Mechnikov, while working in the town of Messina (on the island of Sicily) observed involution of the tadpole's tail via absorption of its muscle cells by the adjacent cells, i.e., the final stage in the life cycle of "superfluous" cells [1]. However, he interpreted the phagocytosis phenomenon as a kind of organism's defense against pathogens, and for this work he was awarded in 1908 the Nobel Prize (together with Paul Ehrlich). Only about 100 years later, in 1972, John Kerr (University of Queensland) with colleagues summarized their observations on a special form of cell degradation and phagocytosis unaccompanied by immune reactions and inflammation as the concept of apoptosis [2, 3]. Afterwards, this concept was supplemented with data on a highly organized self-destruction of cells not only under conditions of disease but during the normal development of plant and animal organisms, and this was termed programmed cell death (PCD) [4, 5].

Abbreviations: APC) antigen-presenting cells; ROS) reactive oxygen species; HSP) heat shock proteins; DC) dendritic cells; ChK) chemokine; o-LDL) oxidized low density lipoprotein; MP) phagocytizing cell (in particular, macrophage); NC) necrotic cells; PM) plasma membrane; PCD) programmed cell death; TNF) tumor necrosis factor; PS) phosphatidylserine; PSR) phosphatidylserine receptor; ABCA1) ATP-dependent phospholipid carrier A1; CRP) C-reactive protein; β2-GPI) β2-glycoprotein I; ICAM-3) intercellular adhesion molecule-3; MBL) mannose-binding lectin; MER) receptor tyrosine kinase; MGF-E8) milk-fat globule epidermal growth factor E8 (lactaderin); MIP-2) macrophage inflammatory protein; PGE_2) prostaglandin E_2; pS) protein S; SP) surfactant proteins; SAP) component of serum amyloid protein; TGF-β) transforming growth factor; TLR) Toll-like receptor.

* To whom correspondence should be addressed.

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For a long time studies on the elimination of "unwanted" cells were focused mainly on the stage of cell death [6], while mechanisms responsible for the control of tissue purification from fragments of the dying cells remained unstudied [7]. Only during the last 4-5 years has significant progress been achieved in this field due to experiments on *Caenorhabditis elegans*, and specific features of phagocytosis of apoptotic cells have been elucidated in many biochemical and genetic details. But the clearance of cells after necrotic death is still poorly studied, although, in contrast to immunologically unnoticeable apoptosis, necrosis of cells is an immunogenic and rather dangerous event.

The present review considers the literature describing mechanisms of recognition and removal of cells dying through apoptosis (form I of PCD) and necrosis (oncosis, necrosis-like PCD) which is specified by excretion of intracellular components (hydrolases, DNA, and other potential immunogens) into the extracellular space and accompanied by inflammation and injury of the surrounding tissues, i.e., autoimmune reaction. Special attention will be given to the role of heat shock proteins as signaling, costimulating, and antigen-presenting molecules in the interaction of necrotic cells and the immune system.

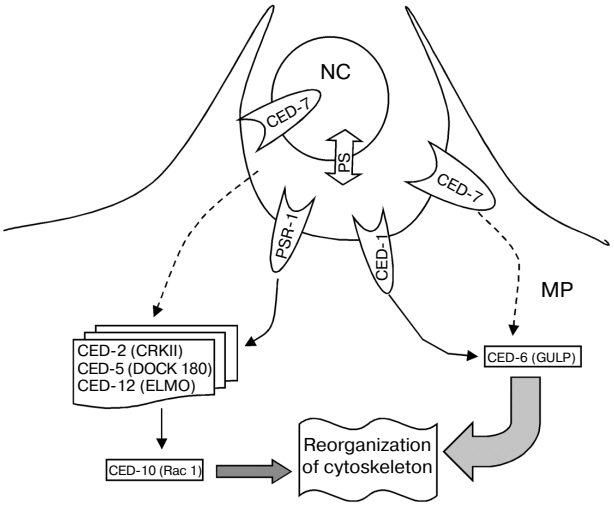

Fig. 1. Phagocytosis of necrotic neurons in *Caenorhabditis elegans*. CED-1, a homolog of mammalian endothelial cell receptor SREC; CED-2 (CRKII), CED-5 (DOCK 180), CED-6 (GULP), and CED-12 (ELMO), adaptor proteins of the nematode and their homologs in mammals; CED-10 (Rac-1), GTPase of the Rho family; PSR-1, phosphatidylserine receptor in *C. elegans*.

CLEARANCE OF "UNWANTED" CELLS IN *Caenorhabditis elegans*

The nematode *Caenorhabditis elegans* seems to be the most genetically demonstrative model of an organism's purification from corpses of "unwanted" cells, although biochemical features of this process are far from clear.

Even in the early 1990s, in *C. elegans* the genes *DEG-1* and *MEC-4* were found, which were specified by dominant mutations responsible for necrotic death of some inserted and mechanosensor neurons during embryogenesis [8, 9]. The neurons died on the 500th min after the zygote's first division because of over-activation of Na^+-channels containing a product of the mutant *mec-4(u231)* gene. Analysis of this model revealed that the necrotic cells were removed by cells of the surrounding hypodermis with involvement of the same genes as in phagocytosis of apoptotic cells (Fig. 1).

One of the mechanisms involves products of three genes. CED-1, which is a structural homolog of the SREC receptor (of the SR-F family found on human epithelial cells), seems to recognize phosphatidylserine (PS) exposed on the dying cell surface and through the adaptor CED-6 (an analog of the engulfment adaptor protein (GULP) of mammals) transmit the signal for reorganization of the cytoskeleton to provide for phagocytosis. The signal is translated to CED-6 also with involvement of CED-7 (a protein of the ATP-binding phospholipid carriers [10]). The receptor link of another mechanism, in addition to the receptor of the anionic phospholipid phosphatidylserine, also involves other yet unidentified molecules [11]. This pathway maintained by a complex of adaptor proteins starts with CED-2 (a homolog of the mammalian CRKII) and includes CED-10 (a homolog of Rac GTPase of the Rho family).

Although mechanisms of phagocytosis of apoptotic and necrotic cells are similar, their kinetic characteristics are considerably different. Thus, necrotic cells are absorbed for about 1 h longer than apoptotic cells [12]. It is unclear whether this is associated with the difference of signals initiating phagocytosis on the surface of the corresponding cells and/or presence of additional anchored signaling molecules. In any case, the reduced expression of phagocytosis genes during the elimination of necrotic cells (which starts later than the elimination of apoptotic cells) and their size several times greater than the size of normal cells slow down the phagocytosis. Note that apoptosis can also be accompanied by cell swelling [13]. The protein calreticulin, which functions as a chaperon and Ca^{2+} depot in the endoplasmic reticulum, is believed to be a possible signal of necrotic cells. Knockout of the calreticulin gene was associated with suppression of necrotic death of neurons induced by the mutant gene *mec-4(u231)* [14].

Two significant characteristics of elimination of "unwanted" cells in *C. elegans* should be also mentioned that so far have been described only for phagocytosis of apoptotic cells.

1. To realize PCD, the cells adjacent to the cells to be eliminated have to possess full-value ability of phagocytizing. Thus cells that should have been eliminated sur-

vived in *C. elegans* embryos with mutant genes of phagocytosis (*ced-1*, *ced-6*, and *ced-12*). However, these cells displayed transient (fluctuating) signs of PCD (cytoplasmic and nuclear condensation, decrease in the refraction of nuclei) [15].

2. An exceptional accuracy in the recognition of "unwanted" cells. Some such cells are phagocytized even before the termination of the mitotic division resulting in such cells [12].

Unfortunately, studies on this object very suitable for genetic analysis meets a limitation because the nematode lacks a specialized immune system with its own mechanisms similar to the system developed during evolution from osseous fishes to mammals for recognizing cells to be eliminated.

PROGRAMMED ELIMINATION OF APOPTOTIC CELLS IN ANIMALS

Cells of various tissues in a multicellular organism undergo continuous elimination during both development and the adult state and are replaced with new cells from the stem reserve. Some such cells, e.g., those of the intestinal epithelium (about 200 g per day in humans) and of the skin surface are simply released into the external medium (exfoliation). However, in other parts of the body "unwanted" cells pass an obligatory preliminary stage determined by the apoptotic program of destruction, which acts similarly in nematodes and mammals. In 2002, a Nobel Prize was awarded for the deciphering of the fundamental genetic mechanisms of this program [16]. Such cells in lymphoid organs, vascular epithelium, blood, and other tissues are phagocytized by the adjacent cells or professional macrophages. It is surprising that the phagocytes, which degrade the intracellular components, fail to process them as antigens and do not provoke the development of "anti-self" immune reaction.

Even in 1967, some stages of phagocytosis of cells in mammals were described [17] that were uniformly manifested during both normal processes (morphogenesis, cellular metabolism, atrophy, etc.) and under pathologic conditions (ischemia, trauma, burn, etc.) (Fig. 2).

1. Apoptotic destruction of a cell makes it ready for being adhered and absorbed by a phagocyte. This is associated with expression on the cell surface of ligands (PS, oxidized PS (o-PS), annexin-I, ICAM-3, etc.), which signal to the phagocyte: "eat me" [18-20]; moreover, chemoattractants for macrophages lysophosphatidylcholine [21] and thrombospondin-1 [22] are also secreted. Unfortunately, at present data on these signals involved in PCD are insufficient [23]. To initiate apoptosis, at least under physiological conditions (neurogenesis in *C. elegans* and mice), phagocytes are required, which can somehow select "unwanted" cells from a group of uniform cells [24, 25].

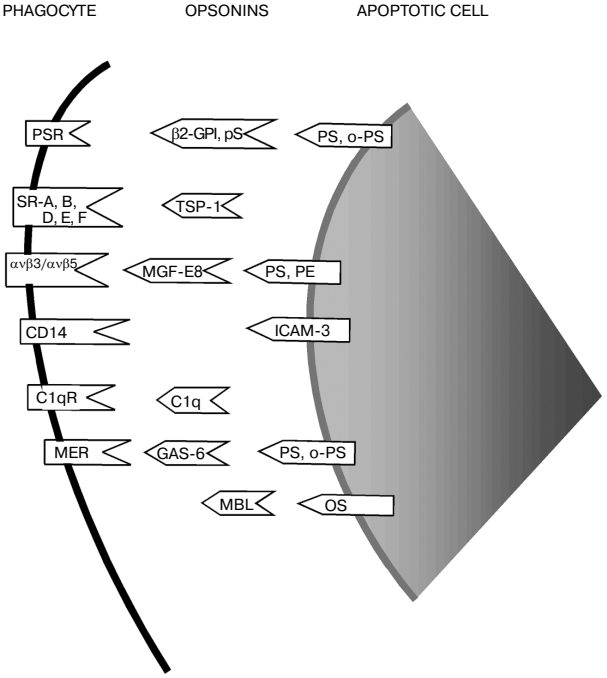

Fig. 2. Mediators of phagocytosis of apoptotic cells in mammals. Molecules on the plasma membrane surface of phagocyte: PSR) phosphatidylserine receptor; SR) receptors with lipoproteins as a ligand; αvβ3/αvβ5) vitronectin receptors; CD14) component of the lipopolysaccharide-binding complex; C1qR) receptor of the C1q complement component; MER) receptor tyrosine kinase. Opsonins: β2-GPI) β2-glycoprotein I; pS) protein S; TSP-I) thrombospondin-I; MGF-E8) milk-fat globule epidermal growth factor E8 (lactaderin); GAS-6) growth arrest specific gene 6 factor; MBL) mannose-binding lectin. Ligands of apoptotic cells: PS) phosphatidylserine; o-PS) oxidized phosphatidylserine; OS) oligosaccharides; PE) phosphatidylethanolamine; ICAM-3) intercellular adhesion molecule-3.

2. A significantly higher number of molecular mediators of phagocytosis are presented on the surface of phagocytizing cells, in particular, macrophages. These mediators include lipoprotein receptors (scavenger receptors SR-A, -B, -D, -E, -F), pathogen-associated molecular pattern receptors CD14 and TLR [10], but phosphatidylserine receptor (PSR) is studied better than the others [26].

3. In addition to molecules bound to the surface of interacting cells, phagocytosis is also contributed by the so-called opsonins, or a family of soluble proteins promoting the adhesion of an "unwanted" cell to the phagocyte and the structural rearrangement of the latter. Opsonins include the complement system components (C1q, C3, C3b, iCb3), immunoglobulins (IgG, IgM), collectins (MBL, SP-A, SP-D), acute phase proteins or pentraxins (CRP, SAP, pentraxin-3), an anticoagulant pS, the growth arrest specific gene 6 (Gas-6) – a pS-homologous protein, β2-glycoprotein [27-29]. Note, that some opsonins (iCb3, C1q, MGF-E8 (milk-fat globule

epidermal growth factor E8), and thrombospondin-1) are secreted by macrophages [30].

4. Finally, the fourth component of the system for elimination of "unwanted" cells is a complex of enzymes catalyzing degradation of corpses of the absorbed cell and cleaving protein antigens into short peptides. Just during this phase, antigen-presenting cells (APC) (i.e., macrophages and dendritic cells) can either maintain the organism's tolerance to its own antigens or induce the cellular and humoral immune response [31]. Under physiological conditions (embryogenesis, immunogenesis, renewal of epithelium and other tissues), apoptotic cells are phagocytized silent for the surrounding tissues [32], although APC obviously meet their own intracellular antigens (e.g., nucleosomes [33]). To explain this reaction of the phagocytes, a hypothetical mechanism of providing tolerance to self antigens has been proposed. This mechanism is based on the data that the phagocytes with absorbed apoptotic cells release anti-inflammatory mediators (TGF-β, PGE_2), which suppress the development of immune reaction against self antigens [34]. Apoptotic cells themselves also secret tolerance-providing cytokines [35]. The interaction of PS with its receptor resulting in inhibition of signaling functions of pro-inflammatory receptors of the TLR type seems to be one of the initial links in this mechanism [36]. However, this hypothesis was not confirmed by experimental data. The *in vitro* pro-inflammatory reaction was found not only during phagocytosis of apoptotic cells [37-40], but also on the contact of intact cells with dendritic cells (DC) [41]. Tolerogenicity/immunogenicity of apoptosis is likely to depend on the stimulus initiating the destruction. Thus, apoptotic death of cells of some cell lines of human mammary gland carcinoma induced with anti-DNA-HSP70 was accompanied by inflammation and secretion of TNF [39]. Target cells were also phagocytized before manifestations of apoptosis [42-44] and did not induce the anti-inflammatory activity of phagocytes [45].

Redox processes play a dual role in the absorption of PS-exposing cells. On one hand, the treatment of apoptotic cells with H_2O_2 inhibits the absorption and this suggests the presence of phagocytosis factors sensitive to oxidation [46], and on the other hand, phagocytes do not bind with apoptotic cells in the absence of oxidized PS [47].

It is difficult to interpret the above-described findings because the models under study are multicomponent: the population of target cells concurrently includes intact, apoptotic, and necrotic cells. Obviously, without an additional purification and isolation of these subpopulations even their minor amounts in the total cell culture will influence the efficiency of phagocytosis and immunogenicity of antigens presented by the phagocyte [48, 49]. Moreover, it should be also kept in mind that exposition of PS is not always a signal of cell destruction [50], and, vice versa, cell destruction can occur without this marker [20, 47].

Thus, the molecular picture of the above-described phagocytosis and processing of antigens is very far from complete and rather contradictory, as compared to the apoptosis stage, which precedes the stage of cell elimination. This is not only because of the complexity of the phenomenon that involves two objects, the cell to be removed and the phagocyte, but also due to existence in mammals, including humans, of the highly differentiated and specialized immune system which plays a decisive role in purification of the organism from fragments of dying cells. The system designed for discriminating the "self and non-self" and clearance of the "non-self" is also involved in elimination of the "self" as "unwanted" cells, and this is associated with the tolerance to the organism's own antigens [23, 51].

PHAGOCYTOSIS OF NECROTIC CELLS

In contrast to apoptotic cells with their content being isolated before being phagocytized, necrotic cells present a powerful inflammatory and immunogenic stimulus [32, 52]. Their intracellular components and later cell remains, which are released across the damaged plasma membrane, are a source of endogenous adjuvants, intracellular antigens, attractants, and activators of neutrophils, phagocytizing and dendritic cells, and also PCD-initiating factors [53, 54]. However, recent studies have shown these processes to be more sophisticated that they were earlier believed. Numerous data indicate that necrosis, similarly to apoptosis, is realized as a controlled and genetically programmed process [14, 55-58]. And similarly to apoptosis, necrotic destruction is suggested to be accompanied by appearance of specific markers, chemokines, and phagocytosis promotors that modulate phagocytosis and immunogenic reaction of APC.

Ligands for phagocytosis. In many cases, appearance of PS on necrotic cells is an early and sufficient event for phagocytosis, which occurs even before perforation of the cell membrane [63, 64]. During this so-called pre-necrotic period, the disturbance of asymmetric distribution of aminophospholipids in the plasma membrane generally follows the same rules as in apoptotic cells.

The role of PS in the absorption of the lysed cell remains was mainly studied on the model of metabolically induced necrotic destruction (Table 1) where the PS exposition depended on energy metabolism [20, 59] and functioning of caspases, which could be activated within 1 min (e.g., in hypoosmotic shock [65]). However, as soon as the plasma membrane lost its integrity, PS was very rapidly exposed on its outer layer, and this was not associated with the increased entrance of Ca^{2+} into the lysed cell [66].

Table 1. Role of necrotic stimulus type and the plasma membrane state in the efficiency of absorption of necrotic cells by phagocytes

Line of target cells	Necrotic stimulus	Plasma membrane state	Efficiency of phagocytosis**	Line of phagocytes	References
T-lymphoma Jurkat*	STS + oligomycin + (–) glucose	intact, PS (–)	low***	MM*	[59]
		permeable, PS ↑	high****		
	STS + z-VAD.fmk	intact, PS (–)	high		
	ionomycin	intact, PS ↑	high		
T-hybridoma DO11.10	55°C, 15 min	permeable, PS ↑	high low	J774A.1 RAW 264.7	[60]
Burkitt lymphoma JLP-119, BL-41	H_2O_2, 75 μM	permeable	high	MM*	[61]
Mouse fibrosarcoma L929sAhFAS	TNF	permeable, PS ↑	high	Mf4/4	[62]

Notes: MM, macrophages resulting by cultivation of blood monocytes; PS ↑, phosphatidylserine is exposed; PS (–), phosphatidylserine is not exposed; STS, staurosporine; z-VAD.fmk, pancaspase inhibitor.
* Human cells.
** Efficiency of phagocytosis was assessed by the number of absorbed cells per phagocyte and/or the percentage of phagocytizing cells.
*** Comparable with phagocytosis of intact cells.
**** Comparable with phagocytosis of apoptotic cells.

The role of a molecular carrier ABCA1 (a homolog of the nematode's Ced-7) in phagocytosis of the removed cells is less clear. It is suggested that this carrier is involved in the displacement of PS from the outer to the inner layer of the plasma membrane, and a decrease in the ABCA1 activity can promote disorders in the lipid asymmetry of the membrane. This is supported by data on ABCA1 functioning as a carrier of cholesterol and phospholipids to protein receptors of the apoA-I type during formation of lipoproteins. Therefore, on a decrease in the ATP content, which is characteristic for necrotic destruction, this ATP-dependent carrier is inactivated and the PS content on the outer surface of the plasma membrane increases as a result of its passive diffusion [10, 67].

The exposure initiating cell death influences rather ambiguously the rate of phagocytosis and the number of cells absorbed by a single phagocyte [43]. Necrotic cells with disorders in energy or Ca^{2+} metabolism were absorbed similarly to apoptotic cells. Effective absorption of necrotic cells obtained on inhibition of caspases and not exposing PS suggested that, in addition to PS, other ligands of the "eat me" type should also exist [59]. In an artificially mixed population of apoptotic and necrotic cells, macrophages preferred the necrotic cells [66]. However, the necrotic cells obtained under conditions of heat shock did not compete with apoptotic cells for macrophages [60]. The necrotic cells obtained through receptor-mediated death were absorbed very efficiently. Although phagocytosis in this model started only after the plasma membrane had been perforated and PS exposed, its rate and intensity became characteristic for phagocytosis [62].

These data (Table 1) indicate significant difficulties in creation of adequate models describing phagocytosis of necrotic cells. Histological data show necrosis as a phenomenon that involves large populations of cells, contrastingly to apoptosis which concerns individual cells [68]. This type of the cell destruction seems to be determined by the combination of several causes: the thanatogenic activity of some factors (including reactive oxygen species (ROS)) released by necrotic cells and destroying the adjacent cells, the low efficiency of absorption of necrotic cells by the surrounding nonprofessional phagocytes (e.g., because of their size [12] and suppression of phagocytosis by ROS [61]), and a poor mobilization of macrophages. These hypotheses seem rather reasonable not only for the so-called post-mitotic tissues in the case of ischemia (brain, kidney, heart), but also for lymphoid organs where regions of necrotic cells appear under conditions of viral infection (HIV-1 [69, 70]) and autoimmune disease (systemic lupus erythematosus [71]).

Experiments with the integrin-binding tetrapeptide RGDS and PS-containing liposomes have shown that, depending on the cell type, not only PS but also vitronectin receptors contribute to the recognition and absorption of necrotic cells by phagocytes [60, 66], and this suggests common features in phagocytotic mechanisms of necrotic and apoptotic cells. Electron microscopy of absorption of necrotic cells has shown its morphology to be reminiscent of micropinocytosis [72], whereas phagocytosis of apoptotic cells goes by a "zipper" mechanism [26].

Opsonins. A number of serum proteins are required for effective phagocytosis of necrotic cells, and these proteins seem also to diminish pro-inflammatory consequences of the process. Lymphocytes (56°C, 30 min) and granulocytes (to a lesser degree) bound the complement components C3 and C4 immediately on addition of necrotic cells into the culture medium, as differentiated from apoptotic cells, which bound these proteins during the post-apoptotic stage when their plasma membrane became permeable [73]. Serum components C1q and DNAse-1 [74], SAP [75] promoted the utilization of chromatin from the lysed cells, and this seemed to prevent the development of autoimmune reaction. Mannose-binding lectin, which is usually secreted by activated monocytes, can also be an opsonin of necrotic cells. This lectin was adsorbed on necrotic cells (56°C, 30 min) similarly to the case of late apoptotic cells with permeable plasma membrane [27]. An acute phase protein pentraxin-3 induced in tissues during inflammation was also involved in the clearance of necrotic cells [76].

Necrosis is an immunogenic death. The above-presented data suggest that phagocytes recognize and absorb necrotic cells using the same apparatus that eliminates apoptotic cells. But why does the presence of the marker PS on necrotic cells not prevent the pro-inflammatory reaction [19]? Studies on mechanisms of phagocytosis of both necrotic and apoptotic cells are associated with difficulties in the preparation of a highly purified population of the cells dying through a definite pathway. To study immunogenic properties, necrotic cells are usually prepared by homogenization, heating to 55-56°C, or freezing–thawing three-to-five times. Obviously, the targets prepared for phagocytosis in such a way are reservoirs containing intracellular antigens which can be easily released and, thus, may be used as models of some extreme exposures, such as burns, frostbite, squeezing, etc. Table 2 presents examples that suggest activation of macrophages and dendritic cells by necrotic cells and their content. This activation is accompanied by inhibition of secretion of anti-inflammatory cytokines (IL-10, TGF-β) and by release of pro-inflammatory mediators (TNF-α, IL-1β, IL-6, MIP-2, IL-8) and chemokine. Exposed to the factors of necrotic cells, dendritic cells enter the mature state that is characterized by appearance of specific markers (CD40, CD80, CD86), costimulating molecules (B7.1, B7.2), presence of the main histocompatibility complex of absorbed antigens, stimulation of T-cell proliferation, etc. Immunization of animals with dendritic cells loaded with appropriate necrotic tumor cells suppressed the growth of tumors.

The necrotic cells obtained by exposure to some antitumor drugs also acquired immunogenicity. Embryonic fibroblasts knocked-out by pro-apoptotic genes and treated with a toxic dose of a DNA-alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) induced the secretion of TNF-α by macrophages [83]. Melanoma cells incubated with a viral fusion-inducing membrane glycoprotein (FMG) and dying after the fusion as multiple syncytia induced an effective presentation of a marker antigen of DC [82]. Necrotic cells prepared by treatment with the antitumor drug Gancyclovir also stimulated the *in vitro* pro-inflammatory response of macrophages, and injection of these cells into animals caused a strong antitumor reaction suppressing the tumor growth [81]. An interesting model of rat line intestine cancer with cells expressing cytochrome *c* antisense DNA was studied both *in vitro* and *in vivo*. This tumor displayed an increased necrotic sensitivity to antitumor drugs *in vitro* and immunogenic death *in vivo*; therefore, this tumor could not be retransplanted [84].

Thus, necrosis induced with physical and chemical agents is an immunogenic form of cell death. A model of receptor-induced necrosis was also studied; it had no such features. The cells of mouse fibrosarcoma overexpressing Bcl-2 and dying under the influence of TNF were phagocytized similarly to apoptotic cells, and their cytokine profile did not change [62].

Pro-inflammatory mediators and receptors. A search for pro-inflammatory mediators released by necrotic cells unexpectedly resulted in a discovery not of new molecules but new functions of well-known polypeptides, including heat shock proteins [85] (Fig. 3).

These highly conservative proteins, such as the inducible Hsp70, constitutive Hsp60, gp96, and calreticulin, are released from necrotic cells and very effectively promote the presentation of intracellular antigens of APC [79, 86, 87]. Note that not only physical destruction of the cells resulting in a damage to the plasma membrane but also biological exposures initiate the release of heat shock proteins. An extract from roots of *Licania michauxii* Prance (order *Rosacea*) induced necrotic destruction in various lines of tumor cells, and this was accompanied by an increase in the content of Hsp70 and a 40-fold increase in the content of Hsp70 mRNA [88]. A similar induction of Hsp70 was observed in tumor cells dying necrotically under the influence of Gancyclovir [89].

Data have accumulated suggesting that the important role of HSP in the regulation of thanatogenic programs is determined not only by their chaperon activity [90-92]. The activity of many chaperons depends on the

Table 2. Immunogenic properties of necrotic cells

Necrotic factor	Target cells (released factors)	Phagocytes	Pattern of immune reaction	References
F/T, osmotic shock	Kidney adenocarcinoma* 293	DCM*	Induction of DC maturation. DC-stimulated proliferation of T-cells	[77]
Mechanical homogenization	MEF, thymocytes	DCBM*, MEF	IL-12, ChK (iRNA)↑, NFκB↑	[78]
F/T	E.G7	DCM	MHCII↑, B7.1↑, B7.2↑, CD40↑	[79]
F/T	B16-BL-6 melanoma	DCM	CD40↑, CD80, CD86, MHCII↑, IL-12↑, antitumor effect of DC immunization	[49]
F/T	Neutrophils*, T-cells Jurkat*	MP*	MIP-2↑, IL-8↑, TNF-α↑	[80]
55°C, 25 min	T-hybridoma DO11.10	RAW 264.7	TNF-α↑, IL-6↑	[60]
Gancyclovir	CMT93tk-bcl-2	IC-21	IL-10↓, TGF-β↓, TNF-α↑, IL-β1↑, IL-6↑	[81]
FMG	Melanoma*: Me1624(HLA-A2+); Me1888(HLA-A2–)	DCM*	Gp100↑	[82]
MNNG	EF, EF(Bax$^{-/-}$,Bak$^{-/-}$), EF(p53$^{-/-}$)	MBM	TNF-α↑	[83]
TNF	L929s-AhFAS	MP Mf4/4	(–)TNF, (–)IL-6	[62]

Notes: DCBM) dendritic cells from culture of bone marrow; DCM) dendritic cells from culture of monocytes; F/T) freezing/thawing; MBM) macrophages from culture of bone marrow; MM) macrophages from culture of monocytes; MNNG) N-methyl-N′-nitro-N-nitrosoguanidine; FMG) fusion-inducing membrane glycoprotein; MEF) mouse embryonal fibroblasts; ChK) chemokine to mobilize neutrophils; Gp100) melanoma-specific antigen.

* Human cells.

↑/↓ Increase/decrease in secretion or expression, respectively.

(–) Without changes.

following cycle of reactions: binding of ATP, its hydrolysis, metabolism of nucleotides, and association–dissociation of HSP and its substrate. The ATP-bound HSPs have a low affinity for the produced polypeptide, whereas ADP-bound proteins form a stable complex. The cell necrosis induced with various agents is characterized by a considerable decrease in the production and content of ATP, and just these conditions are favorable for appearance of highly immunogenic complexes of HSP and polypeptides. The structure of these complexes is determined by HSP, which promote an effective transmission of antigens to APC [93]. Thus, the activation of chaperons not only provides for deaggregation and correct arrangement of protein complexes produced under conditions of cell stress, but chaperons also act as intercellular signals which, similarly to cytokines, have their receptors [94]. And in the role of "danger signals" HSP may be called shokines, i.e., mediators transmitting information about shock (Fig. 4).

Some receptors have been identified on macrophages that interact with chaperons and stimulate the pro-inflammatory reaction. CD91 (an α-2-macro-globulin/low density lipoprotein-related receptor protein) has been called a sensor of necrosis [95]. Through this receptor, heat shock proteins Gp96, Hsp70, Hsp90, and calreticulin released from necrotic cells transmit the bound necrotic cell antigens to APC to be effectively presented by the main histocompatibility complex [93, 96]. Receptors of the TLR group seem also to be involved in the binding of chaperons [78, 97]. These receptors belong to the IL-1 receptor family (IL-1R) and through an adaptor protein MyD88 (myeloid differentiation factor) and IL-1R-associated kinase (IRAK) transmit the signal to the known transcriptional factor NF-κB. The activity of this factor is determined by the ratio of reductants (e.g., glutathione) and oxidants (e.g., H_2O_2) in the cell [98, 99]. CD40 specific for dendritic cells is also involved in the binding of Hsp70 [100]. A new Hsp70-like immunogenic

protein Hsp70L1 has also been discovered in these cells [101].

Irregularly built polypeptides and their aggregations (β-amyloid, α-sinuclein, prion, tau, polyglutamine-containing polypeptides) which seem to be produced because of inadequate functioning of chaperons and ubiquitin–proteosomal system and are specific for some neurodegenerative diseases can possibly act as immunogenic signals [102]. Intra- and extracellular toxicity of these proteins and their complexes (early precursors of plaques and filaments) can sustain pro-inflammatory conditions [103, 104]. Thus, β-amyloid can directly induce the apoptotic destruction of cells [105].

Protein HMGB1 of chromatin occurred to be a powerful pro-inflammatory factor. This protein is passively released by necrotic cells, whereas in apoptotic cells it closely binds to chromatin during apoptosis [106]. The receptor RAGE (receptor for advanced glycation product) is its acceptor on APC [107].

Other components of apoptotic cells are likely to exist, but chemical binding of them inside the dying cell prevents dangerous consequences. In particular, just such a role is played by activation of transglutaminase, an enzyme producing interprotein cross-links and thus strengthening the plasma membrane, which promotes generation of isolated apoptotic bodies [108].

Among other macromolecules penetrating across the perforated plasma membrane and activating DC mRNA is a possible ligand for TLR3 [109]. The blood plasma content of hypomethylated DNA, which can be accepted by TLR9, is increased in systemic lupus erythematosus [110]. Fragments of nucleosomal chromatin resulting because of cell degeneration and capable of initiating necrosis in adjacent cells can also be immunogenic signals of necrotic cells [111].

Proteases and their inhibitors can be also considered intracellular components modifying the anti-inflammatory reaction of APC to exposed PS. In particular, elastase of neutrophils can cleave the PSR and, thus, inhibit the transmission of an anti-inflammatory signal through

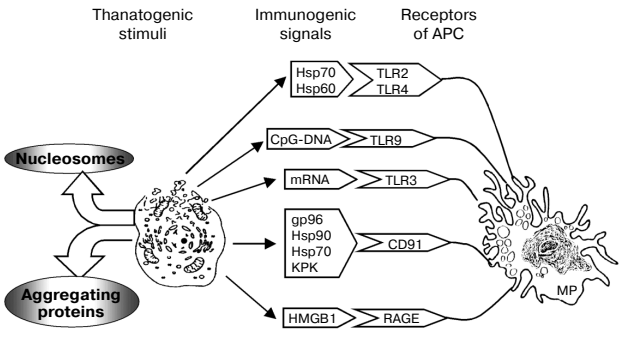

Fig. 3. Molecular mediators, their receptors, and immunogenicity of necrotic cells.

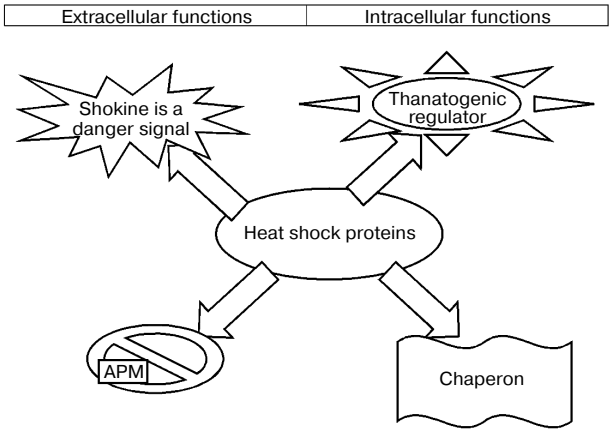

Fig. 4. Possible cellular roles of heat shock proteins in the mechanisms mediating the immunogenicity of necrotic cell destruction. APM) antigen-presenting molecules.

this receptor and releasing of the corresponding mediators [29, 80].

And another unique molecular signal should be mentioned which is released from lysed cells (post-apoptotic or necrotic) and acts as a costimulator and adjuvant for other pro-inflammatory mediators [112]. The content of the purine catabolite uric acid increases in the damaged cells, and its microcrystals stimulate the maturation of dendritic cells and T-response to antigen in culture. The uric acid content in tissues is near saturation; therefore, even a slight increase in it as a result of synthesis induced by a damaging factor easily causes over-saturation and crystallization. It is possible that this crystallization–dissolving of uric acid can be used in the organism as an indicator of danger [113].

Interest in the interaction of apoptotic and necrotic cells with the innate and adaptive immune systems of mammals and humans, in particular, has quickened in the past few years [114]. On one hand, this is associated with the further development of concepts about immunological recognition of "self and non-self" (F. M. Burnet and P. B. Medawar, the 1960 Nobel Prize [115]). The organism's response to pathogens is likely to depend not only on their invariant molecular structures but also on the danger signals released by the damaged cells [116]. Studies on mechanisms of APC reaction to necrotic death of cells significantly contributes to extrapolation of this hypothesis onto mechanisms of interaction of tissues damaged as a result of a pathological process [112, 117].

The detection of the necrotic elimination of "unwanted" cells in inflammatory, degenerative, autoimmune, ischemic, and cancerous human diseases supports the urgency of such studies for development of more effective approaches for their treatment [55, 118-124]. Cell therapy of tumors seems promising by vaccination of

patients with dendritic cells grown from autogenic bone marrow and cultured in the presence of the lysed tumor tissue [125-127] and under conditions promoting the induction of chaperons [128, 129].

When this paper was being prepared for printing, an extremely interesting hypothesis appeared concerning a new principle of antitumor therapy, namely, about the possibility to stimulate phagocytosis of tumor cells by their neighbors and/or macrophages without a cytotoxic exposure [130]. For those who are interested in the problem of immune response to cell death a fine review should be mentioned which specially considers the clearance of apoptotic cells [131].

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