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Review

Secondary necrosis: The natural outcome of the complete apoptotic program

Manuel T. Silva *

Instituto de Biologia Molecular e Celular, University of Porto, Rua do Campo Alegre 823, 4150-180 Porto, Portugal

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ABSTRACT

The predominant definition of apoptosis considers that the elimination of the apoptosing cell is by heterolytic degradation following phagocytosis by an assisting scavenger (efferocytosis). However, an alternative and largely underestimated outcome of apoptosis is secondary necrosis, an autolytic process of cell disintegration with release of cell components that occurs when there is no intervention of scavengers and the full apoptotic program is completed. Secondary necrosis is the typical outcome of apoptosis in unicellular eukaryotes but, importantly, it may also occur in multicellular animals and has been implicated in the genesis of important human pathologies. Secondary necrosis is a mode of cell elimination with specific molecular and morphological features and should be considered the natural outcome of the complete apoptotic program.

1. Introduction

Active cell death processes follow suicidal, genetically regulated, programs that make use of an endogenous molecular machinery [1]. Presently accepted forms of typical active cell death include apoptosis, necrosis (or necroptosis [2]), autophagic death and cornification [3].

Cell death is always followed by cell elimination by cytolytic disintegration with loss of cell's identity. The initial description of apoptosis [4] considered that the elimination of the apoptotic cell was by heterolytic degradation following phagocytosis by an assisting scavenger cell. The term efferocytosis was coined for the removal and degradation by scavengers of apoptotic corpses [5]. Several cell types are able to phagocytose apoptotic cells but the cells of the myeloid phagocyte system [6], mainly macrophages, are the prime phagocytes for this task [7]. The heterolytic degradation of the apoptosing cell in the scavenger phagosome [8] superimposes on the autonomous proteolytic degradation progressing in the apoptosing cell as part of the apoptotic program [9] and results in passive necrosis of the phagocytosed apoptotic cell (heterophagic necrosis) [8,10,11].

The outcome of apoptosis assisted by an auxiliary scavenger cell is a result of the expression at the surface of apoptosing cells of apoptotic cell-associated molecular patterns (ACAMPs) [12] that phagocytes of the multicellular organism recognize as "eat-me" signals [13,14]. The most universal and best characterized "eat-me" signal is the translocation of phosphatidylserine to the outer leaflet of the lipid bilayer, which occurs very early in the apoptotic process [15]. Besides the "eat-me" signals, the interaction between apoptotic cells and scavenger cells uses bridging molecules [13,14] and "find-me" signals [16,17], as well as absence of "don't eat-me" signals that prevent the phagocytosis of viable cells [18].

Elimination of the early apoptotic cells by scavenger cells is the norm in the typical course of physiological apoptosis in multicellular animals, where it is a mechanism of cell deletion operating in cell populations and tissues to regulate cell numbers [4]. This view of apoptosis has become predominant in the literature and is the only one considered in the recommendations on the use of cell death-related terminology proposed by the Nomenclature Committee on Cell Death (NCCD) [3,19].

However, a central issue in the apoptotic process is that, when scavengers do not operate, the apoptotic process proceeds to an autolytic necrotic outcome. This autolysis was called secondary necrosis by Wyllie et al. [20] intending to distinguish this mode of cell elimination from "cellular necrosis occurring ab initio", which should be called "primary necrosis" [21] (reviewed in [22]) (Table 1).

The present review will now discuss results which suggest that secondary necrosis is the natural outcome of the complete apoptotic program at all levels of eukaryotic organization and that efferocytosis interrupts that program before apoptosing cells attain secondary necrosis. Efferocytosis represents an assisting process used by multicellular animals as a strategy to avoid the deleterious consequences associated to the progression of apoptosis to autolytic secondary necrosis.

* Fax: +351 226 099 157.
E-mail address: mtsilva@ibmc.up.pt

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Table 1
Features of pre-necrotic apoptosis [4,20], secondary necrosis [27,35], and primary necrosis [22].

	Apoptosis
Pre-necrotic apoptosis	
	Cell rounding and shrinking
	Nuclear fragmentation (karyorrhexis)
	Intense chromatin condensation (pyknosis)
	Oligonucleosomal DNA fragmentation
	Near-to-Intact cytoplasmic membrane
	Phosphatidylserine externalization
	Intact lysosomal membranes
Secondary necrosis[a]	
	Karyorrhexis
	Pyknosis
	Release of activated caspase-3
	Cytoplasmic swelling
	Lysosomal membrane permeabilization
	Cytoplasmic membrane permeabilization
	Primary necrosis
	No nuclear fragmentation
	Moderate chromatin condensation
	Cell swelling
	Lysosomal membrane permeabilization
	Cytoplasmic membrane permeabilization

[a] Apoptotic and necrotic features are in bold and in italic, respectively.

2. Secondary necrosis is the natural outcome of fully developed apoptosis in multi- and unicellular eukaryotes

2.1. Secondary necrosis in multicellular animals

Besides resulting in cell death, apoptosis leads to cell elimination. Cell elimination is the opposite of mitosis and, like mitosis, the apoptotic process of cell elimination should primarily be viewed as a single cell process. However, the predominant characterization of apoptosis in multicellular animals implies that the apoptotic mode of cell deletion is a two-cell process as it involves the doomed cell and the assisting scavenger. To analyze the unfolding of the complete apoptotic process of metazoan cells apoptosis has to be studied in cells apoptosing without the assistance of scavengers. One approach is to analyze in vitro the progression of apoptosis in metazoan cells that have been isolated from the social context of the organism and subjected to an apoptogenic stimulus as was early done by John F. Kerr's group. In those studies using murine lymphocytes [23] or a mammalian lymphoblastic cell line [24] cultured in vitro, apoptosis was seen to progress until a terminal disintegration by secondary necrosis. Since then, whenever in vitro kinetic studies on the progression of apoptosis in non-phagocytic metazoan primary cells or cell lines were carried out, thus using conditions where scavenging by phagocytosis of apoptotic cells is not interfering, the outcome of full developed apoptosis was secondary necrosis (see, for example, the studies with mammalian primary cells [25] or immortalized cell lines [26,27]). Cultured metazoan cells apoptosing in vitro express "eat-me" signals [28,29] and, if co-incubated with macrophages, they are phagocytosed [30,31] mimicking the vivo physiological apoptosis.

Apoptosis follows an intrinsic, genetically controlled, program [1]. The molecular dissection of this program shows that apoptogenic stimuli activate in in vitro cultured metazoan cells a sequence of molecular events that, when uninterrupted, proceed to cell disruption by secondary necrosis [27,32]. In vitro and in vivo, the full apoptotic program comprises two phases (upper path in Fig. 1). In the first (pre-necrotic) phase the apoptosing cell maintains cytoplasmic membrane integrity, and molecular alterations largely due to activated caspases [33] produce the classical apoptotic phenotype (Table 1 and Fig. 2B). It is during this phase that the externalization of "eat-me" signals occurs. The second is a necrotic phase (secondary necrosis) with cytoplasmic swelling, rupture of the plasma membrane and terminal cell disintegration with release of intracellular components (Fig. 2C) following a sequence of molecular events that have been described in detail [27,29,34,35].

Progression of apoptosis to secondary necrosis can be observed in vivo in situations where clearance by scavengers does not operate and the complete apoptotic program fully evolves. (i) This is the case with some physiological situations where apoptotic cells are shed into ducts or into territories topologically outside the organism (like the gut or the airways lumen) or in the lumina of the acini, where the chances of encountering scavengers are small [36,37]. (ii) The protein tyrosine kinase Mer is one of the phagocyte receptors engaged in linking the apoptotic cell to the scavenger phagocyte [38]. Mer knockout mice have macrophage defects which result in deficient clearance of apoptotic cells [38,39] and accumulation of secondary necrotic cells was observed [39]. In *Caenorhabditis elegans* mutation in genes required for phagocytosis of apoptotic cells results in the progression of many of these cells to secondary necrosis [40,41]. (iii) Moreover, extensive secondary necrosis has been described in multicellular animals in situations of massive apoptosis that surmounts the available scavenging capacity [42–44], and when this capacity is directly affected by detrimental effects on scavenger cells [45–48] or by processes that affect molecules involved in phagocytosis of apoptotic cells by scavenging cells [39,49].

The above observations support the conclusion that the complete apoptotic process in multicellular animals is genetically controlled by an intrinsic program that includes an autolytic termination by secondary necrosis, which makes that process self-sufficient leading to self-elimination when scavengers are not available. The above observations also show that there is an obvious and important functional limitation associated with efferocytosis: depending on an assisting scavenger cell, this mechanism fails if that cell is not available or is defective in which case apoptosis will proceed to its completion, leading to the secondary necrotic outcome. Importantly, as in the data above reviewed, this failure and the consequent occurrence of secondary necrosis in vivo is typically due to defects in, or lack of, scavengers, that is to mechanisms independent from the apoptotic program which is running to completion.

Apoptotic bodies may be formed during the degradation phase by the fragmentation of apoptosing cells as already described in the initial report of Kerr and collaborators [4]. Although formation of apoptotic bodies is frequently considered as the rule during apoptosis, in some otherwise typical apoptotic processes occurring in vitro or in vivo apoptotic bodies are not produced or are rare [9,20], so that the elimination of apoptotic cells frequently affects non-fragmented cells. Like apoptotic cells, apoptotic bodies are initially enveloped by a membrane with retained integrity, express "eat-me" signals, and are phagocytosed by scavengers [4] or undergo secondary necrosis if not cleared [20].

2.2. Secondary necrosis in unicellular eukaryotes

The proposal of the term apoptosis [4] culminated a series of studies carried out with metazoan tissues and the disclosing of essential aspects of the molecular machinery of this mode of cell elimination, and of its genetic control, again was done using multicellular animals mainly *C. elegans* and *Drosophila* [50,51].

The timeline of our knowledge about the apoptotic mode of cell elimination explains why the concept of apoptosis was initially linked to multicellularity and was not envisaged as a single cell event. Since the first description of physiological cell death was

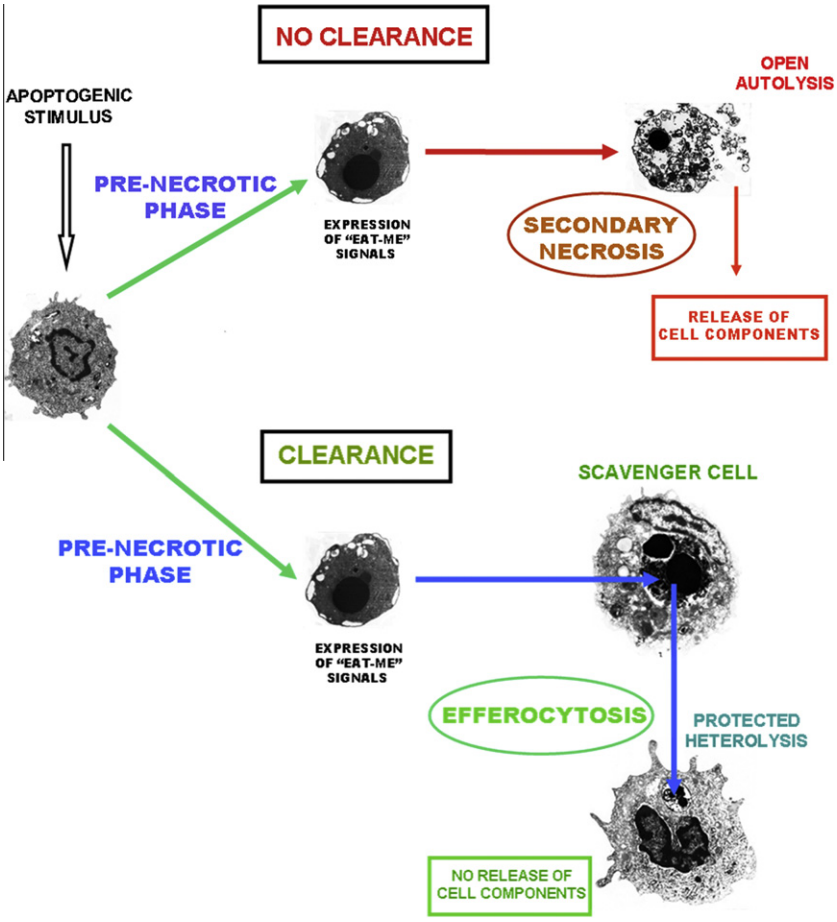

Fig. 1. The two pathways of apoptosis in multicellular animals. Apoptosis in multicellular animals may follow two alternative paths that lead to quite diverse elimination processes, respectively depending on being affected (CLEARANCE) or not (NO CLEARANCE) by phagocytosis of the apoptosing cell by a scavenger. The two paths share a first common pre-necrotic phase (green arrows) during which the dismantling of the doomed cell is initiated by autonomous degradation but with conserved cytoplasmic membrane integrity, and both paths terminate by cytolysis. In the absence of clearance, the pre-necrotic phase progresses to secondary necrosis (red arrows) and the terminal cytolysis is autolytic as it is carried out from within by the autonomous proteolytic process under way in the apoptosing cell. This means that the complete apoptotic program comprises a final autolytic step (secondary necrosis). In apoptosis assisted by a scavenger cell (blue arrows), the normal progression of apoptosis is interrupted by the incorporation of the apoptosing cell within a phagosome of the scavenger and the terminal cytolysis is by heterolysis: the apoptosing cell is dismantled by the degradative proteolytic activity of the scavenger which induces passive necrosis of the phagocytosed apoptotic cell (efferocytosis). Cytolysis in secondary necrotic cells occurs openly and releases potentially dangerous cellular components (OPEN AUTOLYSIS), while in assisted apoptosis cytolysis occurs in the safe niche of a phagolysosome of the scavenger that ingests the doomed cell when it still is in the pre-necrotic phase of apoptosis, thus preventing the release of the cell contents (PROTECTED HETEROLYSIS). Although with diverse consequences, expression of "eat-me" signals during the pre-necrotic phase occurs in the two paths of apoptosis. The upper path leading to secondary necrosis is the rule in unicellular eukaryotes. Adapted from Fig. 1 in Ref. [104].

made by Vogt in 1842 (quoted in [52]) until the first descriptions of apoptosis in unicellular eukaryotes in 1994 [53], all studies on cell death modes regarded metazoans. The initial description of the apoptotic mode of cell elimination, based on observations with mammalian tissues [4], helped to promote the metazoan-centric perspective that such mechanism was exclusive of multicellular animals. When performing physiological roles in these organisms apoptosis includes efferocytosis and is a silent and useful process which is crucial during embryonic morphogenesis and, in adult life, in tissue homeostasis and immune responses for the elimination of unnecessary, unwanted or dangerous cells [54], thus perfectly fitting multicellularity.

More than two decades elapsed after the proposal of the term apoptosis and before cell death with apoptotic features started to be recognized in unicellular eukaryotes. Apoptosis was then accepted as a process not exclusive of multicellular animals but rather as a universal mechanism of cell elimination operating according to a basic program not only in men, mice, worms and flies but also in the simpler and more ancient forms of single-celled eukaryotes [53,55].

Indeed, cell death processes with typical apoptotic features have progressively been reported in unicellular eukaryotes in all major eukaryotic groups from the Opisthokonta (that includes metazoans) to the more distant Discicristata (Table 2). When kinetic studies of membrane permeability were carried out in the listed examples of apoptotic processes in unicellular eukaryotes, secondary necrosis was found to be the outcome (Table 2), as in the case with metazoan cells apoptosing in vitro.

It is relevant that externalization by apoptotic cells of "eat-me" signals, namely phosphatidylserine, the most ubiquitous one in multicellular animals [13,14], occurs not only in the case of these eukaryotes where it fulfils the crucial role of providing the recognition by scavengers of apoptotic cells as "unwanted self" that must be removed [56], but also in unicellular eukaryotes where that role is not operating. Studies with unicellular eukaryotes where apoptosis was found to be accompanied by externalization of phosphatidylserine regard, among others, *Dictyostelium discoides*, *Chlamydomonas reinhardtii*, *Plasmodium berghei*, *Blastocystis hominis*, *Leishmania donovani*, *Leishmania infantum*, *Trypanosoma brucei* and *Trypanosoma cruzi* (Refs. in [57]). Importantly, yeasts,

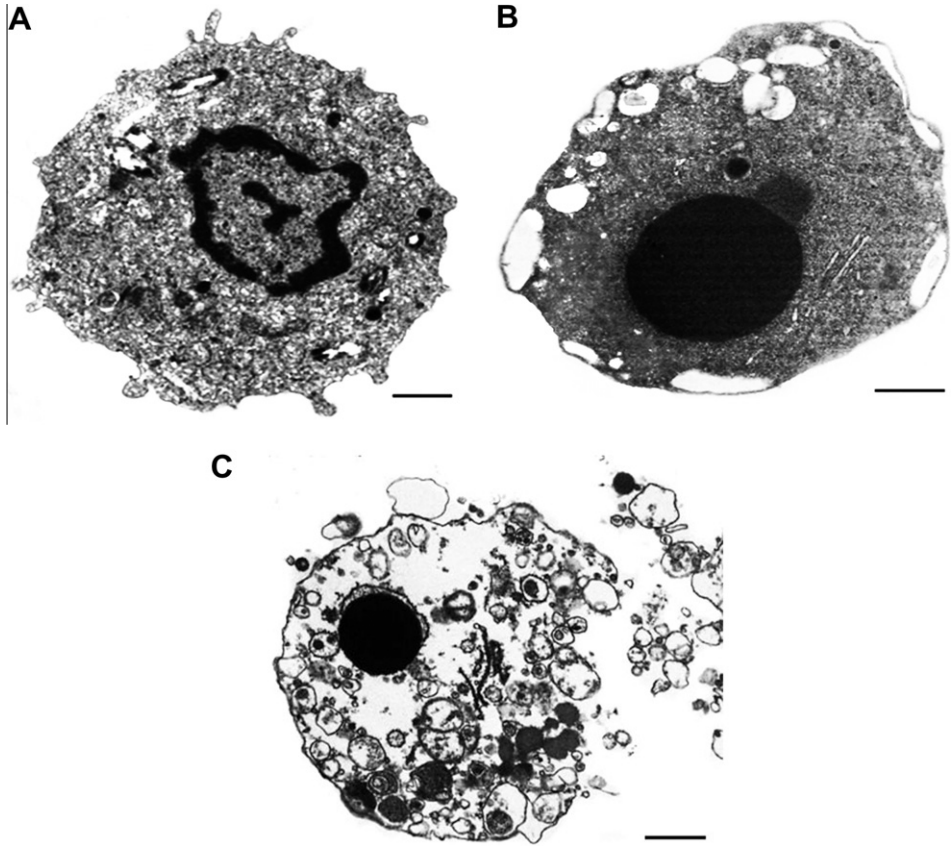

Fig. 2. In vivo secondary necrosis in a vertebrate. Transmission electron microscopy of peritoneal sea bass macrophages following the i.p. inoculation of the bacterial fish pathogen *Photobacterium damselae* subsp. *piscicida* that secretes the exotoxin AlP56 with selective apoptogenic activity towards macrophages and neutrophils [123]. Bar = 0.75 μm. Technical details in Ref. [124]. (A) A normal macrophage. (B) A macrophage under apoptosis, showing cell shrinkage, dense cytosol, intense chromatin condensation and a continuous cytoplasmic membrane. (C) A macrophage under terminal secondary necrosis identified by the co-existence of an apoptotic marker (hypercondensed nuclear fragment) and necrotic features (clear cytosol and rupture of the cytoplasmic membrane with extensive cell disintegration).

Table 2
Examples of eukaryotic unicellular organisms where secondary necrosis was found as the natural outcome of apoptosis

Eukaryotic group[a]	Organism	References	
		Apoptotic markers	Occurrence of secondary necrosis
Opisthokonts	Yeasts	[125]	[126]
Amoebozoa	*Dictyostelium discoideum*	[127]	[128]
Plants	*Dunaliella viridis*	[57]	[129]
	Chlamydomonas reinhardtii	[57]	[67]
Alveolates	*Plasmodium berghei*	[57]	[130]
Heterokonts	*Blastocystis hominis*	[131]	[132]
Discicristates	*Leishmania donovani*	[133]	[134]
	Trypanosoma brucei	[135]	[136]
	Trypanosoma cruzi	[55]	[137]

[a] The listed groups represent six of the eight eukaryotic groups considered by Baldauf [58]. It is of note that the Opisthokonts comprise fungi (including yeasts) and multicellular animals.

the unicellular eukaryotes closest to metazoans [58], also externalize phosphatidylserine during apoptosis. This was found in *Candida albicans* [59,60], *Schizosaccharomyces pombe* [61] and *Saccharomyces cerevisiae* [62–65]. Externalization of phosphatidylserine also has been found in apoptotic cells of higher plants where phagocytosis by scavengers cannot operate [66,67]. Moreover, mammalian phagocytes ingest viable, non-apoptotic cells expressing externalized phosphatidylserine [68–70], as well as necrotic cells expressing that "eat-me" signal [71]. Additionally, it has been shown that *C. elegans* uses the same set of engulfment genes for removal of both apoptotic and necrotic cells [72]. Thus, the recognition and ingestion by phagocytes of cells through the detection of phosphatidylserine is not a mechanism specific of clearance of apoptotic cells.

The above observations suggest that externalization of molecules that are recognized by phagocytes as "eat-me" signals appeared as a by-product of the endogenous degradative process of apoptotic cell death before the emergence of multicellularity and without the theological purpose of promoting the quick and safe clearance of apoptotic cells by scavenger phagocytes before progression to secondary necrosis. This feature of apoptosis would have been maintained during evolution and, in multicellular

animals, phagocytic cells might have learned to read the exposition of "eat-me" signals as a signal for phagocytosis and elimination. Clearance by scavengers of apoptotic cells is a crucial feature of physiological apoptosis in multicellular animals and has been observed at all levels of multicellularity, including in the simplest forms like hydra [73]. Accordingly, those organisms refined the process of removal of apoptotic corpses through the use, as mentioned above, of several redundant "eat-me" signals besides phosphatidylserine as well as of bridging molecules and of "find-me" signals, together with the elimination of "don't eat-me" signals, an indication of the extreme importance of efferocytosis in the context of animal multicellularity.

Thus, the complete apoptotic process, with its secondary necrotic outcome, is an ancient molecular program common to uni- and multicellular eukaryotes. As in isolated metazoan cells, the apoptotic death of a unicellular eukaryote, once triggered, is self-sufficient and leads to self-elimination by secondary necrosis.

3. In multicellular animals avoidance of secondary necrosis through efferocytosis is advantageous

When part of a multicellular animal, a cell that disintegrates by secondary necrosis during in vitro solitary apoptosis will respond to an apoptotic stimulus again by activating its endogenous apoptotic program. However, the outcome will typically be different from that occurring when the cell is isolated because now the apoptosing cell is part of the social context of the multicellular organization [74] and the early expression of "eat-me" signals at its surface promotes the removal by phagocytes of the doomed cell before progression to secondary necrosis. The primary function of apoptosis in multicellular animals is "controlled cell deletion" [4] and this is achievable through secondary necrosis. However, elimination of apoptotic cells by heterolytic efferocytosis instead of autolytic secondary necrosis has crucial advantages to multicellular animals. These advantages include increased speed of cell elimination and more extensive degradation of the apoptotic cell components [8,10,11] and energetically profitable reutilization of phagocytosed and digested components of apoptotic cells allowing for the recycling of molecules [4,75]. Moreover, and most importantly, elimination by efferocytosis occurs while the apoptosing cell still is enveloped by a plasma membrane with retained integrity, and cell disintegration, including permeabilization of the membrane, takes place in the safe recess of the phagolysosome of scavenger cells (protected heterolysis; Fig. 1). This prevents secondary necrotic autolysis which occurs openly (open autolysis; Fig. 1) and thus is potentially pathogenetic as it releases partially degraded pro-inflammatory and immunogenic cell components as will be discussed in the next section.

Therefore, the acquisition of the ability to recognize and remove pre-necrotic apoptosing cells as structures that must be cleared before the completion of the apoptotic program represented a crucial step forward for multicellular animals and conferred to physiological apoptosis in these organisms a particular characteristic dependent on efferocytosis.

4. In multicellular animals secondary necrosis may have relevant medical implications

Although, as mentioned, secondary necrosis in multicellular animals can occur in vivo in particular physiological situations, typically it represents an event with pathological implications because of the frequently deleterious effects of exposition and release of cell constituents [7,28,43,76].

One mechanism for the genesis of pathogenic consequences of extensive or persistent apoptotic secondary necrosis is the leakage of cytotoxic, pro-inflammatory and immunogenic molecules by the autolysing cells. These molecules include damage-associated molecular patterns (DAMPs) [77]. The impact of the release of these molecules on the inflammatory and immune responses depends on the context in which cell death occurs [78], and it may be beneficial as will be discussed below in regards to immunogenic cell death in tumors. However, necrosis-associated release of DAMPs often has pathological pro-inflammatory and immunogenic consequences [28,78,79], contributing to the pathology frequently associated with extensive or persistent secondary necrosis. Release of lysosomal proteolytic enzymes and the oxidative burst occurring during secondary necrosis may participate in the generation of DAMPs by modifying original cell components which are later released due to the eventual rupture of the plasma membrane [27]. Among pro-inflammatory and immunogenic DAMPS released by secondary necrotic cells are proteases, nucleosomes consisting of double-stranded DNA and histones, S100 calgranulin proteins, high mobility group box-1 (HMGB-1) protein, proteolytically processed autoantigens, and urate crystals [26,28,78,80,81]. Nucleosomes produced during the apoptotic death process by internucleosomal DNA degradation may persist until secondary necrotic cell disintegration and be released [82] participating in the genesis of autoimmune disorders [81,83]. Nucleosomes are present in increased amounts in the circulation of patients with systemic lupus erythematosus (SLE) [84].

Secondary necrotic cells can be phagocytosed by scavengers in vivo following molecular mechanisms that have recently been reviewed [85]. That phagocytosis is promoted by "eat-me" signals including ACAMPs exposed during the apoptotic pre-necrotic phase [12] and by modified cell components altered by proteolysis and oxidative events occurring in the necrotic phase and made accessible by plasma membrane rupture [27,85]. Therefore, the process of clearance of early (pre-necrotic) and late (secondary necrotic) apoptotic cells are not identical and usually have diverse consequences in terms of inflammatory and immunogenic responses. While typically the clearance of early apoptotic cells by scavenger phagocytes is anti-inflammatory [31,86], phagocytosis of secondary necrotic cells often is pro-inflammatory and immunogenic and thus may represent another mechanism for the genesis of pathogenic consequences of secondary necrosis [13,85].

In vivo neutrophil lysis due to secondary necrosis is particularly pathogenic [43,76] as these phagocytes are recruited in very high numbers to inflammatory sites [6] and are extraordinarily rich in cytotoxic, tissue damaging molecules [87,88]. One highly cytotoxic phagocyte molecule released by secondary necrotic cells is neutrophil elastase [89,90]. Extracellular active neutrophil elastase was found in several infections where neutrophil secondary necrosis is implicated as a pathogenicity mechanism [48,49,91].

Secondary necrosis may therefore produce acute and chronic pathology, thus being a mechanism for apoptosis turning pathogenic. Secondary necrosis has recently been implicated in an increasing number of human clinically important situations of acute and chronic inflammation with apoptotic cytopathology including many autoimmune disorders [92,93], ischemia [94], atherosclerosis [95,96], chronic obstructive pulmonary disease (COPD) [97–99], lung inflammation associated with oxidative stress including in smokers [100], cystic fibrosis [49,101], asthma [102], bronchiectasis [103], and infection [104].

On the other hand, secondary necrosis affecting tumor cells has recently gained an additional relevance due to its recognition as a process with likely beneficial implications in anticancer therapies by facilitating the activation of the immune system and consequently the clearance of tumor cells. Anticancer chemotherapy [105] and radiotherapy [106] are largely mediated by apoptosis. It has been reported that, after treatment with some chemotherapeutic agents or ionizing irradiation, tumor cells may become

highly immunogenic when injected into immunocompetent mice (reviewed in [107]). Several observations have been accumulated suggesting that such immunogenicity is associated to the progression to secondary necrosis of therapy-induced apoptotic tumor cell death and to the release by secondary necrotic tumor cells of the DAMP molecule HMGB-1: (i) tumor cells treated in vitro with some apoptogenic chemotherapeutic agents or radiotherapy release HMGB-1 protein, and secondary necrosis was suggested to be the mechanism for that release [108]. (ii) As discussed elsewhere [109,110], clearance of apoptotic cells may be insufficient during therapy-induced apoptotic tumor cell death in vivo, resulting in the accumulation of secondary necrotic cells. (iii) It has been found that secondary necrotic tumor cells stimulate DCs [111–113], and thus are immunogenic [110]. The presentation of tumor antigens by mature DC leads to CD4+ and CD8+ T cell activation. HMGB-1 released by secondary necrotic tumor cells is recognized by DCs which prime CD4+ and CD8+ T cells and thereby trigger immunogenic T helper 1 cell and cytotoxic T lymphocyte responses, respectively [80,108,114]. Although less efficiently than macrophages [115] DCs may engulf apoptotic cells, mainly when extensive apoptosis overwhelms the macrophage availability [116] or in territories where DCs outnumber macrophages [117].

5. Identification of cells under secondary necrosis

Necrotic cells seen in cultures or in vivo may represent cells that are being affected by apoptotic secondary necrosis or, instead, by primary necrosis, and there is the possibility of a misinterpretation of the cell elimination process involved in the genesis of the necrotic cells. There are many examples in the literature of studies describing inducers of cell death as triggers of primary necrosis based on experiments where the death process behind that necrosis is apoptotic. This misinterpretation occurs when the lack of adequate kinetic assessments prevents the observation of the pre-necrotic phase of apoptosis and only reveals a necrotic outcome wrongly labeled as primary necrosis. And this is not an issue of academic interest; rather, the correct classification of cell death into specific modalities may be extremely important including in medical situations. Thus, methods for the correct identification of cells under secondary necrosis are important and have been described in detail elsewhere [35,104,118]. As secondary necrosis affects cells that have gone through particular apoptotic alterations, secondary necrotic cells exhibit a specific morphotype (Table 1) that associates apoptotic features, like hypercondensed chromatin (pyknosis) and nuclear fragmentation (karyorrhexis), and necrotic features, like rupture of the cytoplasmic membrane (Fig. 2). For the same reason, cells under secondary necrosis, contrary to primary necrotic cells, release activated caspase-3 [118,119] which can be detected in vivo [48,83,120].

6. Concluding remarks

Apoptosis is a genetically regulated form of active cell death that uses machinery intrinsic to the cell and represents an ancient molecular mechanism common to all levels of eukaryotic organization. The apoptotic program comprises two events which provide two alternative courses with diverse mechanisms of cell elimination: (i) early exposure of surface signals that, in multicellular animals, allow scavenger phagocytes to recognize apoptotic cells as unwanted self. These cells are removed before the completion of the apoptotic program and are dismantled through heterolysis by the scavenger, meaning that this course of apoptosis includes the burial of the dead cell (efferocytosis) as the final part of the process. This is the outcome that makes physiological apoptosis a silent cell elimination process in multicellular animals, the one initially described when the term apoptosis was introduced [4], and the one considered in the recommended definition of in vivo apoptosis [3,19,121]. (ii) Secondary necrosis which, in the absence of scavengers, is activated at the end of the full apoptotic program and leads to the autolytic disintegration of the doomed cell [20].

As efferocytosis does not operate with unicellular eukaryotes, secondary necrosis is the natural outcome of apoptosis in these organisms. For the same reason, secondary necrosis is the rule when in vitro cultured non-phagocytic cells from multicellular organisms undergo apoptosis. However, importantly, secondary necrosis also occurs in vivo in multicellular animals in some physiological and, more frequently, pathological situations when functional scavenger cells are not available and the apoptotic program fully unrolls. Under a broad biological perspective encompassing uni- and multicellular eukaryotes, secondary necrosis is the natural conclusion of the complete apoptotic program, exhibits particular molecular and morphological features and represents a specific mode of cell elimination. The typical course of physiological apoptosis in multicellular animals, as originally described by Kerr and colleagues [4] and predominantly considered in the literature, therefore involves a deviation from the apoptotic program preventing its completion. The definition of apoptosis as a process which terminates with the removal and disintegration of the apoptosing cell by a scavenger, as is predominant in the literature, represents a metazoan-centric perspective and, even specifically considering metazoans, is restrictive. Contrasting with the abundance of studies on apoptosis and on the clearance of apoptotic cells by scavengers, the study of, and reference to secondary necrosis has been largely neglected, reflecting that predominant definition. For example, recent and important texts with recommendations on the use of cell death-related terminology proposed by the NCCD [3,19] and on the use and interpretation of assays for monitoring cell death in higher eukaryotes [122] do not mention secondary necrosis. Further research on secondary necrosis will certainly reveal new instances where this mode of cell elimination has a pathogenetic role or new possibilities for the improvement of therapeutic interventions.

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