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The death of a cell can be defined as an irreversible loss of plasma membrane integrity and represents the final point in the cell life. There are three basic forms of cell death: necrosis (type III cell death), apoptosis (type I cell death) and autophagy (type II cell death). Until recently, the prevalent view was that cellular necrosis is the consequence of non-specific cell injury without underlying signalling events. Over the past decade, it has become evident that in certain conditions, necrosis is the result of a strictly regulated interplay of signalling events, which are initiated by a diverse range of stimuli, supporting the idea that necrotic cell death is as well controlled and programmed as caspase-dependent apoptosis. Thus, depending on the context necrosis might be fully unregulated or, on the contrary, programmed. In the present work, we describe, from a global point of view, an approximation to the necrosis.

Keywords cell death, necrosis, delayed cell death

1. Introduction

The death of a cell can be defined as an irreversible loss of plasma membrane integrity [1]. Historically, three types of cell death have been distinguished in mammalian cells by morphological criteria: necrosis (Type III cell death), apoptosis (Type I cell death) and autophagy (Type II cell death). Necrosis is often defined in a negative manner as death lacking the characteristics of the type I and type II processes. A classical positive definition of necrosis based on morphological criteria (early plasma membrane rupture and dilatation of cytoplasmic organelles, in particular mitochondria) [1, 2], can now be refined [3]. In Figure 1 a typical image of a necrotic is showed.

2. Necrosis: An active and passive death

Until recently, the prevalent view was that cellular necrosis is the consequence of non-specific cell injury from trauma, being an accidental and uncontrolled form of cell death lacking underlying signalling events. This might be true for cell death resulting from severe physical damage, such as hyperthermia or detergent-induced cytolysis. Over the past decade, it has become evident that in certain conditions, necrosis is the result of a strictly regulated interplay of signalling events, which are initiated by a diverse range of stimuli [4]. Although the concept of programmed necrosis has existed in the literature for several years, several recent papers have added significantly to our understanding of the mechanism behind, and the regulation of, this process. The idea that necrosis constitutes a (or even the) default cell death pathway is supported by the observation that inhibition of essential apoptotic events plus inhibition of autophagy can induce necrosis [5]. Moreover, accumulating evidence supports that necrotic cell death is as well controlled and programmed as caspase-dependent apoptosis [6], and that it may be an important cell death mode that is both pathologically and physiologically relevant, suggesting the existence of caspase-independent cell death pathways that

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can function even in a strictly regulated developmental context [7]. Caspase-independent cell death can provide a backup suicide mechanism if the classical apoptosis machinery fails [8, 9]. Cell death induced under such conditions lacks the typical features of apoptosis and instead resembles necrosis [10-12]. Thus, necrotic cell death is not the result of one well-described signalling cascade but is the consequence of extensive crosstalk between several biochemical and molecular events at different cellular levels [13]. Depending on the context necrosis might be fully unregulated or, on the contrary, programmed.

Fig. 2 Necrosis: cell death results from irreversible injury to the cell.

The most remarkable morphological feature of programmed necrosis is the organelle and cell swelling that culminates in the rupture of the plasma membrane (Fig 2). The increase in cell volume and extensive intracellular vacuole
formation implies an imbalance in osmotic pressure [14]. Distinguishing necrosis from apoptosis should never be based on either morphological or biochemical criteria alone, but rather should take into account and integrate all available data [15].

This active necrosis may be executed through a mechanism termed necroptosis or programmed necrosis [16]. Interestingly, necroptosis may be activated upon stimulation by TNFα, FasL, and TRAIL (TNF-related apoptosis-inducing ligand), the same ligands that can activate apoptosis. The major necrotic cell death pathway is mediated through the serine/threonine kinases receptor interacting protein 1 (RIP1) and 3 (RIP3). RIP1 is a crucial initiator of death receptor-mediated necrosis [17] and RIP3 is a crucial upstream activating kinase that regulates RIP1-dependent necroptosis [18-20]. Thus, for example, TNF treatment induces the formation of a RIP1-RIP3 pro-necrotic complex and the kinase activity of both RIP1 and RIP3 is crucial for stable complex formation and subsequent induction of necrosis [18, 20]. Necrostatin-1 was identified as a small molecule inhibitor of necroptosis [15], and more recently, the RIP1 kinase activity was found to be the target of Necrostatin-1 [21].

Therefore, cell death induced by the activation of death receptor may be executed through alternative cell death pathways, apoptosis or necroptosis [22]. Necroptosis is an important cellular death mechanism likely to be involved in many human pathologies from viral infections to neurodegenerative diseases.

3. Cellular events

Necrotic cell death is often associated with pathological conditions being prominent in ischemia, trauma, exposure to toxins and neurodegenerative disorders. Induction of necrotic cell death may be of utmost importance upon, for example, viral or bacterial infection. Extensive DNA damage causes hyperactivation of poly-(ADP-ribose) polymerase-1 (PARP-1) and leads to necrotic cell death [23].

In the event of a HI cerebral aggression several cellular mechanisms are triggered contributing to cell death through apoptosis (Type I of Programmed Cell Death, PCD) or necrosis depending on the severity of damage, the maturative state and region affected [24-27]. Moreover the degree of maturation and the region of the brain affected are also basic factors in the mechanism of damage and cell death. Cell death begins immediately and continues over a period of days or weeks. The phenotype of cell death undergoes a change that ranges from necrotic to apoptotic morphology. This evolution is known as a “necrosis-apoptosis continuum”. Recent studies suggest that apoptosis plays a prominent part in HI aggression in the neonatal brain, apoptosis being more important than necrosis following aggression [28].

ATP levels are an important determinant of whether cells die by apoptosis or necrosis. Necrosis is the end result of a bioenergetic catastrophe resulting from ATP depletion, whereas ATP is required for events preceding apoptotic cell death such as apoptosome formation and caspase activation. Indeed, cells can be rescued from hypoxia-induced necrosis when supplemented with glycolytic substrates [29, 30]. ATP regeneration during reperfusion of ischemic tissues is therefore likely to promote apoptotic cell death over necrotic cell death. Additionally, cells induced to undergo apoptosis instead die by necrosis when ATP synthase is inhibited [31, 32]. Many mediators are involved in the execution phase of necrotic cell death, including reactive oxygen species (ROS), calcium (Ca^{2+}), calpains, cathepsins, phospholipases, and ceramide [33, 34].

4. Pathogenesis and morphological features

In the pathogenesis of cell injury initially there is subletal phase from which a cell is recoverable, or cross a “point of no return” (Fig 3). The permeabilization of mitochondrial membranes determines whether cells will succumb to or survive the injury, and represents a ‘point of no return’ in mitochondrial cell death [35].

From a morphologic point of view [36, 37], the cell exhibits a series of changes that inform us about its stage. Under the light microscope, the early (reversible) changes are difficult to detect, and tissues must be well fixed and stained. It can be observed

- mild cytoplasmic swelling
- dilatation of organelles
- loss of ribosomes and rough endoplasmic reticulum
- blebbing from the plasma membrane of cytoplasmatic fragments, that include cytosol but not the large organellas (mitochondria, endoplasmatic reticulum).

To defined these changes different names are used, such as “hydropic change”, “feathery degeneration”, “cloudy swelling” or “vacuolar degeneration”.

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After an injury a cell can show early (reversible) changes or cross the point of no return. This point is classically associated to the high-amplitude movements in the mitochondria, which imply an important separation between the inner and outer mitochondrial membranes with the consequent irrecoverable loss of oxidative phosphorylation. Cross the “point of no return” an intracellular chaos is displayed, as showed in Figure 2 and 3.

- T cell swells rapidly
- T mitochondrial dilatation
- T high amplitude swelling of mitochondria,
- T appearance of matrix densities (probably represent denatured protein)
- T plasma and internal membranes begin to rupture
- T organelles are found in the extracellular space
- T nuclear structures remain relatively intact. Distinction between euchromatin and heterochromatin is retained, and nuclear pores remain dispersed around the membrane
- T some times presence of deposits of calcium phosphates (particularly in hypoxia)
- T activation of an inflammatory response (Fig 4):

In the last phases of necrosis several events are displayed.

a) at cytoplasmic level:
- T cytoplasm loses detail and acquires a homogeneous eosinophilic appearance (ground glass)
- T mitochondrial swelling
- T irregularities in the cytoplasmic membrane
- T matrix densities and vacuoles
- T deposits of calcium phosphates

b) at nuclear level:
- T chromatin patterns are coarse
- T pyknosis (chromatin condensation)
- T karyorrhexis (chromatin fragmentation)
- T karyolysis (chromatin dissolution)
5. Pyroptosis

Pyroptosis (Fig 5) is a more recently recognized form of regulated cell death distinct from necrosis and apoptosis [38]. Cells dying by pyroptosis have biochemical and morphological features of both apoptotic and necrotic cells [39]. Pyroptosis is commented in the chapter “Cell death. A comprehensive approximation. Delayed cell death” of this same book.
6. Morphological forms of necrosis

When cell death by necrosis occurs in part of an organ or tissue various morphological forms can be observed:

6.1 Coagulative necrosis

In this form, the dominant process is the denaturation of intracytoplasmatic proteins. Although the cells show the signs of nuclear death, the most outstanding trait is the retention of the general architectural pattern of the tissue, despite the death of its constituent elements [40]. Affected cells or tissue are converted into a dry, dull, fairly homogeneous eosinophilic mass as a result of the coagulation of protein.

Coagulative necrosis is characteristic of ischemic injury, usually as consequence of an infarct. In the kidney and in the heart the histological examination of such an area show the “ghost outlines” of the architectural elements of the tissue, although the constituent parenchymal cells are clearly dead. Finally, a slow but progressive ingrowth of connective tissue occurs and eventually the infarct becomes converted to a fibrous scar in which some calcium salts may be deposited (dystrophic calcification) [36].

Characteristically, in the central nervous system, after a hypoxic/ischemic injury, the pattern of necrosis is dominated by enzymatic digestion and subsequent liquefaction of the dead tissue.

6.2 Caseous necrosis

Caseous necrosis is a distinctive form of coagulative necrosis characteristically observed in tuberculosis. The term caseous is derived from the cheesy white gross appearance of the area of necrosis (caseous = cheese-like). On microscopic examination the affected tissue appears completely structureless. The necrotic focus appears as amorphous granular debris seemingly composed of fragmented, coagulated cells and amorphous granular debris enclosed within a distinctive inflammatory border known as a granulomatous reaction. Joint to the coagulated proteins is detected large amount of lipids.

Although the exact mechanisms of liquefaction are unknown, the available data suggest that dysregulated proteolysis, direct mycobacterial toxicity, the Koch phenomenon [41] and Shwartzman reaction [42], and host effector cells and cytokines are key players [43]. Mycobacterial antigens induce the release of cytokines such as tumor necrosis factor (TNF-α), mixed Th1/Th2, and transforming growth factor (TGF-β), collectively facilitate liquefactive necrosis and deranged extracellular matrix turnover [43].

Caseation is also a feature of the epithelioid cell granulomas of tertiary syphilis, known as gummas.

6.3 Liquefactive necrosis

Liquefactive necrosis (or colliquative necrosis) is characteristic of focal bacterial or, occasionally, fungal infections, because microbes stimulate the accumulation of inflammatory cells. In this type of necrosis, the effect of lysosomal enzymes is dominant, with accumulation of protein-rich, semifluid material. The end result is transformation of the tissue into a liquid viscous mass. If the process was initiated by acute inflammation, the material is frequently creamy yellow because of the presence of dead white cells and is called pus.

The pattern observed in the brain after hypoxic/ischemic injury is typically liquefactive rather than coagulative, and in the final stages of this type of necrosis the whole nervous parenchyma is invariably liquefied, resulting in eosinophilic cellular debris [40].

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1023