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Review

Pyroptosis and its relationship to atherosclerosis

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ABSTRACT

Pyroptosis is a pro-inflammatory form of regulated cell death and is dependent on the enzymatic activity of inflammatory proteases that belong to the family of cysteine-dependent aspartate-specific proteases (caspases). Pyroptosis is morphologically, mechanistically, and pathophysiologically distinct from other forms of cell death, including apoptosis and necrosis. Pyroptosis is characterized by rapid plasma membrane rupture, with the consequent release of intracellular contents and pro-inflammatory mediators, including interleukin (IL)-1β, IL-18, and the alarmin HMGB-1.Recent studies have shown that pyroptosis may be involved in atherosclerosis and play an important role in atherosclerotic lesion instability. Here, we review the progress made in understanding the morphological, molecular, and pathophysiological mechanisms of pyroptosis and its potential role in atherosclerosis.

1. Introduction

The incidence of atherosclerosis has increased dramatically in recent decades and has resulted in serious harm to human health. Atherosclerosis is a leading cause of death in developed countries and is characterized by the deposition of fibrous tissues and lipids in the intima of elastic arteries, leading to thrombus formation and structural damage marked by thickening and hardening of the vessel walls [1]. Cell death and inflammation play critical roles at various stages of atherosclerosis. The death of endothelial cells (ECs), macrophages, smooth muscle cells (SMCs), and other types of vascular cells are commonly observed during the development of atherosclerosis. In the early stage of atherosclerosis, dying ECs may release adhesion molecules, pro-inflammatory cytokines, and chemokines to attract monocytes and other inflammatory cells for transendothelial recruitment, and an increase in the proliferation and migration of SMCs into the arterial intima, leading to vasodilator dysfunction and injury [2–5]. Although it was reported that the death of macrophages in early atherosclerotic lesions could decrease the cytopathic effect and inflammatory response, the death of these cells in advanced lesions could promote necrotic core formation and atherosclerotic plaque instability [6,7]. In the course of atherosclerosis, macrophage death is strongly correlated with inflammation [7]. In cases in which dying macrophages cannot be effectively cleared in atherogenesis or plaque progression, the accumulation of these dying cells results in necrotic core formation and release of inflammatory cytokines, chemokines, proteases, and intracellular lipids into the extracellular space, which ultimately triggers inflammation, increases the susceptibility of atherosclerotic plaques to rupture, and forms thrombus [6–8]. The death of SMCs in atherosclerosis may decrease lesion cellularity, weaken the fibrous cap of the plaque, and promote lesion instability [9]. These data indicate that cell death occurs in all major cell types in atherosclerotic lesions and plays a major role in atherosclerosis.

Cysteine-dependent aspartate-specific proteases (caspases) are an evolutionarily conserved class of intracellular proteases [10]. The role of apoptotic caspases (caspases 2, 3, 6, 7, 8, 9, and 10) has been well studied [11]. However, several members of the caspase family have gained prominence as critical mediators of inflammation and innate immune responses and were designated inflammatory caspases [10]. Three human caspases (caspase-1, caspase-4, and caspase-5) and three murine caspases (caspases 1, 11, and 12) have been categorized into the inflammatory caspase subfamily [12]. Sequence analysis suggests that human caspase-1 and murine caspase-1 are orthologues, whereas human caspases 4 and 5 have originated from a duplication of murine caspase-11 [13]. Caspase-1, the precursor of inflammatory caspases, is present in the cell cytosol as pro-caspase-1, an inactive zymogen. The activation of caspase-1 is required for cleavage of pro-interleukin(IL)-1β and pro-IL-18 into mature pro-inflammatory cytokines IL-1β and IL-18, respectively [3]. Some studies have demonstrated the existence of up to 70 protein substrates for caspase-1, most of which play a role in atherogenesis and vascular inflammation [14]. Caspases play central roles in initiating apoptosis and pyroptosis but are not involved in other programmed cell death [15]. Activated caspase-1 can cause a pro-inflammatory form of regulated cell death known as pyroptosis, which is

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characterized by cellular lysis and the release of cytosolic contents to the extracellular space [16], resulting in inflammation. In addition, activated caspase-1 may perform vital functions in atherogenesis.

Cell death and inflammation in atherosclerosis have been extensive investigated. Many research groups have demonstrated the association between cell death and inflammation in atherogenesis, and other groups have hypothesized that apoptosis and impaired efferocytosis—the effective clearance of apoptotic cells—were the major cause of inflammation and death of lesion cells [17,18]. However, recent studies have shown that dying cells in human atherosclerotic plaque undergo cell lysis, but not apoptosis [19–21]. Caspase-3, the primary executor of apoptosis, is rarely expressed in advanced lesions [22]. Of note, plaque rupture is associated with a strong immunoreactivity to caspase-1, and caspase-1 is abundantly expressed in animal and human atherosclerosis and is more strongly correlated with atherosclerotic plaque instability [19,22–24]. Pyroptosis is uniquely dependent on caspase-1 activation [25]. Recent studies have shown that pyroptotic cells can show positive terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) staining, which has traditionally been considered a marker of apoptosis [26,27]. Therefore, increasing evidence has demonstrated that pyroptosis, a caspase-1 dependent cell death, may be involved in atherosclerosis and lesion instability [28].

2. Definition of pyroptosis

The term pyroptosis was described as a pro-inflammatory form of cell death [15,16]. It is derived from the Greek roots “pyro,” meaning fire or fever, and “ptosis,” meaning falling, to describe pro-inflammatory programmed cell death [29]. The first study of caspase-1 dependent cell death used mouse macrophages infected with the Gram-negative bacteria *Shigella flexneri* [30]. In the human host, *Shigella* invades the colonic mucosa, where it infects the phagocytes of the lamina propria, leading to extensive macrophage death and abscess formation [31]. *Shigella* was the first invasive bacteria reported to induce host cell death, which was originally described as apoptosis [32]. Other studies have unraveled a cell death pathway that did not depend on the apoptosis executor caspase-3 but depended on the activity of the inflammatory caspase-1 [30]. The pharmacological inhibition of caspase-1 by acetyl-tyrosyl-valyl-alanyl-aspartyl-aldehyde (Ac-YVAD-CHO) or the genetic ablation of caspase-1$^{-/-}$ in mice increased the resistance of macrophages to *Shigella*-induced cytotoxicity whereas the deficiency of caspases 3 and 11, and *p53* did not induce resistance [30,33,34]. These findings in *Shigella*-infected macrophages were consistent with the results on the induction of caspase-1-dependent cell death by *Salmonella typhimurium* [35–37]. Macrophages are killed when infected with *Salmonella* but are rescued by Ac-YVAD-CHO treatment [35]. More importantly, dying macrophages are insensitive to caspases 3, 6, and 7 [35,38]. Therefore, these findings support the existence of a caspase-1-mediated cell death pathway, which is distinct from apoptosis. To date, caspase-1-dependent cell death has been reported in macrophages infected with several pathogens, including *Listeria monocytogenes* [39], *Legionella pneumophila* [40,41], *Yersinia pseudotuberculosis* [26], *Pseudomonas aeruginosa* [42], *Burkholderia pseudomallei* [43], and *Francisella tularensis* [44].

3. Morphological features of pyroptosis

Cell death is typically discussed dichotomously as either apoptosis or necrosis. Apoptosis is characterized by the active, programmed process of autonomous cellular dismantling without the release of cytoplasmic content to the extracellular space, and the effective inhibition of inflammation [16]. Necrosis has been characterized as passive and accidental cell death; it is triggered by environmental perturbations and results in the uncontrolled release of inflammatory cellular contents [16]. However, recent studies have discovered other forms of cell death, including autophagy, oncosis, necroptosis, and pyroptosis. Pyroptosis is a pro-inflammatory form of cell death. Morphologically, pyroptosis seems to be a combination of apoptotic and necrotic cell death and involves the loss of plasma membrane integrity and the release of cytoplasmic content into the extracellular space [35,38,45]. The plasma membrane in pyroptotic cells appears to rupture, rapidly reseals and swells, and forms a balloon-shaped vesicle around the nucleus [45]. In fact, during pyroptosis, dying cells undergo a measurable increase in size [26,43]. As pyroptotic cells swell, the nucleus undergoes rounding and condensation [40,45]; however, in contrast to apoptosis, nuclear integrity of pyroptotic cells is maintained. Pyroptotic cells undergo DNA fragmentation and, as observed in apoptotic cells, show positive TUNEL staining [26,44,46]. Although the cellular mechanisms that mediate pyroptosis are still unknown, they are dramatically distinct from classical apoptotic pathways. Pyroptosis is triggered by the activation of inflammatory caspases (caspase-1 or murine caspase-11) [47]. In contrast, the caspases involved in apoptosis, including caspases 3 and 6, do not participate in pyroptosis [34,35]. In apoptotic cells, poly-ADP ribose polymerase (PARP) is cleaved by main caspases to preserve cellular ATP energy stores [48]. Although PARP is also cleaved by caspase-1, PARP does not significantly influence pyroptosis using PARP inhibitors or in PARP$^{-/-}$ macrophages [26,49,50]. During apoptosis, internucleosomal DNA cleavage occurs by the activation of caspase-activated DNase (CAD) and degradation of the inhibitor of CAD (ICAD) [51]. More importantly, mitochondrial outer membrane permeabilization in apoptotic cells releases apoptogenic factors into the cytosol, including cytochrome *c* and Smac/DIABLO [52]. However, DNA laddering and ICAD degradation do not occur during pyroptosis [27,53]. Moreover, mitochondrial integrity is maintained, and cytochrome *c* is not released in pyroptotic cells [27,38,39] (Table 1).

4. Molecular mechanisms of pyroptosis

Inflammasomes (also known as pyroptosomes) are multimeric protein complexes that assemble in the cytosol after recognizing pathogen-associated molecular patterns (PAMPs) derived from invading pathogens, danger-associated molecular patterns (DAMPs) induced by

Table 1
Pyroptosis is distinct from apoptosis.

	Characteristics	Pyroptosis	Apoptosis	References
Morphology	Cell lysis	✓	✗	35,38,45
	Cell swelling	✓	✗	27,43,45
	Pore formation	✓	✗	26,35,43
	Membrane blebbing	✗	✓	45
	Membrane-enclosed apoptotic bodies	✗	✓	16
	“Balloon-shaped” vesicle formation	✓	✗	45
	Nuclear condensation	✓	✓	40,45
	Nuclear integrity	✓	✗	40,45
	DNA fragmentation (TUNEL positive)	✓	✓	26,27,44,46
	Mitochondrial integrity	✓	✗	27,38,39
Mechanisms	Caspase-1 activation	✓	✗	25
	Caspase-3 activation	✗	✓	34,35
	Caspase-6 activation	✗	✓	34,35
	Caspase-11 activation	✓	✗	47
	Poly-ADP ribose polymerase (PARP) cleavage	✗	✓	27,48–50
	Inhibitor of caspase-activated DNase (ICAD) cleavage	✗	✓	27,51,53
	Mitochondrial outer membrane(MOMP)/ Cytochrome c	✗	✓	27,38,39,52
Outcome	Inflammation	✓	✗	16