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Review

Cell death and autophagy in tuberculosis

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ABSTRACT

Mycobacterium tuberculosis has succeeded in infecting one-third of the human race though inhibition or evasion of innate and adaptive immunity. The pathogen is a facultative intracellular parasite that uses the niche provided by mononuclear phagocytes for its advantage. Complex interactions determine whether the bacillus will or will not be delivered to acidified lysosomes, whether the host phagocyte will survive infection or die, and whether the timing and mode of cell death works to the advantage of the host or the pathogen. Here we discuss cell death and autophagy in TB. These fundamental processes of cell biology feature in all aspects of TB pathogenesis and may be exploited to the treatment or prevention of TB disease.

1. Introduction

Programmed cell death and autophagy are fundamental processes of cell biology intimately involved in the interaction between *Mycobacterium tuberculosis* (*Mtb*) and the phagocytes it infects, including macrophages, dendritic cells (DC) and neutrophils. The remarkable success of *Mtb* as a human pathogen results from its capacity to evade the innate antimicrobial effector mechanisms of mononuclear phagocytes (MPs) and leverage the intracellular environment as a replication niche. Infected MPs are faced with a pathogen surviving in phagosomes that fail to incorporate the molecular machinery needed to reduce vacuolar pH and generate free radicals of oxygen or nitrogen, and that fail to fuse with lysosomes to expose bacilli to damaging hydrolases [1]. Plan B for the infected MP is to undergo programmed cell death, which eliminates the intracellular sanctuary and exerts other potentially host-protective effects described in Section 3.1.2. Alternatively, a variety of extracellular signals may activate the autophagic machinery of infected MP to drive *Mtb* into lethal autolysosomes as described in Section 5. These responses set the stage for what are now recognized as a very complex series of measures and countermeasures culminating in the survival or death of the infecting pathogen or its host cell, the progression or resolution of immune pathology, and outcome of tuberculosis (TB) disease.

2. Overview of programmed cell death

A requirement for regulated cell death to support tissue development and homeostasis was conceived by Karl Vogt in 1842 but the term apoptosis to describe a morphologically distinct form of non-traumatic cell death and the understanding of its biochemical mechanisms did not emerge until the late 20th century [2]. Apoptosis is a tightly regulated process of cellular deconstruction. It minimizes inflammation and bystander injury by containing the dismembered nuclear and cytoplasmic contents of dying cells within membrane-bound vesicles called apoptotic bodies that are engulfed by other phagocytes in a process called efferocytosis (Section 3.1.2). Binding of apoptotic bodies to specific receptors on MPs responding to “find me” and “eat me” signals induces the expression of anti-inflammatory cytokines including transforming growth factor-β and interleukin (IL)-10 to further insure the silent

Abbreviations: AIF, apoptosis-inducing factor; AMPK, AMP kinase; Apaf-1, apoptotic protease activating factor; BMM, bone marrow-derived macrophages; cFLIP, cellular FLICE-like inhibitory protein; cIAP, cellular inhibitor of apoptosis protein; COX, cyclooxygenase; CTL, cytotoxic T lymphocytes; CYPD, cyclophilin D; DAMP, damage-associated molecular pattern; DC, dendritic cell; FADD, Fas-associated death domain; HrtA2/Ommi, high temperature requirement; IL, interleukin; IMM, inner mitochondrial membrane; LT, leukotriene; LX, lipoxin; MAPK, mitogen-activated protein kinase; MPT, mitochondrial permeability transition; *Mtb*, *Mycobacterium tuberculosis*; mTOR, mammalian target of rapamycin; NET, neutrophil extracellular trap; NK, natural killer; OMM, outer mitochondrial membrane; PG, prostaglandin; PI, propidium iodide; PtdSer, phosphatidylserine; PTP, permeability transition pore; RIPK, receptor interacting serine/threonine protein kinase; Smac, second mitochondria-derived activator of caspases; SLR, SodA, superoxide dismutase; SLR, sequestasome-like receptor; TEM, transmission electron microscopy; TB, tuberculosis; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRADD, TNFR-associated death domain; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end-labeling; VDR, vitamin D receptor.

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elimination of cellular corpses [3,4]. The ultrastructural morphology of apoptosis is characterized by cell shrinkage and chromatin condensation (pyknosis), nuclear fragmentation (karyorrhexis), and blebbing of the outer cell membrane that culminates in apoptotic body formation. Chromosomal DNA is cleaved at internucleosomal boundaries, demonstrated by laddering of DNA bands on gel electrophoresis. Phosphatidylserine (PtdSer), a membrane component that in viable cells is held facing the cytosolic side of the plasma membrane by the enzyme flippase, translocates to the outward-facing surface in apoptotic cells. Exposure of PtdSer on the cell surface plays an important role in membrane stability and clearance of apoptotic bodies (Section 3.1.2).

Necrosis is a much different death, defined by the loss of outer cell membrane integrity with release of cytoplasmic and nuclear contents to the extracellular space. Necrosis was originally thought to result only from accidental events (e.g. freezing or crushing) but regulated mechanisms of necrosis were later identified (Section 2.2) [5]. The ultrastructural morphology of necrosis is characterized by cytoplasmic swelling (onicosis), cytoplasmic vacuolization and swelling of organelles including mitochondria and cell nuclei [6]. These changes result from ATP depletion and the failure of plasma membrane ion pumps to maintain a stable osmotic gradient. Necrosis can also result from direct plasma membrane damage, which disrupts the cells without onicosis. Rupture of the plasma membrane provokes inflammation by releasing damage-associated molecular patterns (DAMPs) such as heat shock proteins, high-mobility group box 1, S100 proteins, extracellular genomic and mitochondrial DNA, ATP, monosodium urate, and heparin sulfate [7,8,8–12]. Binding of DAMPs to their cognate receptors activates an innate inflammatory response and sends "endogenous adjuvant" signals that can stimulate DC to promote T cell activation [9]. The diversity of protein and non-protein DAMPs ensures redundancy in immune stimulation but most converge on common pathways involving inflammasomes, IL-1 and leukotriene (LT)B4 [9,13].

2.1. Apoptosis signaling and execution

Three major pathways of apoptosis initiation (extrinsic, intrinsic and perforin/granzyme) converge on a common execution mechanism with degradation of chromosomal DNA and nuclear and cytoskeletal proteins. Both steps in this process involve caspases; a family of cysteine-dependent aspartate-directed proteases constitutively expressed as zymogens. Caspases operate in a cascade for the rapid induction of apoptosis, which is energy-dependent but independent of transcription [14]. Initiator caspases-8, -9, and -10 are activated by dimerization following recruitment to signaling complexes (Sections 2.1.1 and 2.1.2) [15]. Activated initiator caspases then cleave and activate the pre-formed dimeric zymogens of the executioner caspases-3 and -7. Activation of executioner caspases is necessarily a tightly regulated event but can in some circumstances be mediated by proteases other than initiator caspases.

2.1.1. Extrinsic apoptosis

The extrinsic pathway begins with ligand binding to tumor necrosis factor receptor (TNFR) family proteins containing a death domain in their cytoplasmic tail which serves as the site for signal complex formation [16]. The receptor/ligand pairs most relevant to TB are TNF-α/TNFR1 and Fas ligand/Fas [17,18]. TNF-α binding trimerizes TNFR1 allowing recruitment of TNFR1-associated death domain (TRADD), receptor interacting protein kinase (RIPK)1, TNFR-associated factor (TRAF)2, TRAF5, cellular inhibitor of apoptosis protein (cIAP) 1, and cIAP2 to form membrane-associated complex I. Signals from complex I activate NFκB that upregulates pro-survival genes [19]. Apoptosis is initiated from TNFR1 following dissociation of complex I constituents to form cytoplasmic complexes. Complex IIA contains TRADD, Fas-associated death domain (FADD) and caspase-8. Its formation is opposed by cellular FLICE-like inhibitory protein (cFLIP) that is induced by NFκB [20]. The TRADD-independent complex IIB (also called the ripoptosome) forms when TNFR1 is activated but cIAP1 is inhibited by mimetics of second mitochondria-derived activator of caspases (Smac). Formation of complex IIB also requires deubiquitination of RIPK1 by cylindromatosis. FasL binding to Fas recruits FADD which in turn recruits procaspase-8 and/or cFLIP via death-effector domain interactions. This forms a membrane-associated death inducing signal complex but a secondary cytosolic complex of FADD, cFLIP, and caspase-8 can be released to further amplify apoptosis initiation [19].

2.1.2. Intrinsic apoptosis

The intrinsic apoptosis pathway is induced by diverse intracellular stresses such as DNA damage, starvation, and oxidative stress that lead to outer mitochondrial membrane (OMM) permeabilization. Cytochrome *c* released from the mitochondrial inter-membrane space binds the cytosolic protein apoptotic protease activating factor (Apaf-1) to form a multimeric signaling complex called the apoptosome. The apoptosome recruits and activates procaspase-9, which in turn activates executioner caspases [21]. Smac and the serine protease HtrA2/OMI are also released from the inter-membrane space; they amplify intrinsic apoptosis by relieving the constitutive caspase repression mediated by cIAP family members. Mitochondrial permeability is controlled by Bcl-2 family proteins that either promote or inhibit apoptosis [22,23]. Pro-apoptotic Bax and Bak form pores in the OMM to release cytochrome *c*. This is opposed by anti-apoptotic family members (e.g. Bcl-2, Bclx-L, Mcl-1) but further promoted by other pro-apoptotic Bcl-2 proteins. Cell fate is determined by the integrated activities of pro- and anti-apoptotic Bcl-2 family proteins. Activated caspase-9 cleaves pro-apoptotic Bid into an enzymatically active truncated form (tBid), which orchestrates the activities of Bax to accelerate cytochrome *c* release. Caspase-8 can also cleave Bid, providing a means for crosstalk between the extrinsic and intrinsic apoptosis pathways [24].

2.1.3. Perforin/granzyme mediated apoptosis

A third apoptosis induction pathway is mediated by serine proteases of the granzyme family contained, along with perforin, in granules of cytotoxic T lymphocytes (CTL) and natural killer (NK) cells [25]. Perforin creates pores in the plasma membrane of target cells through which the granzymes are introduced. Granzyme B cleaves the initiator caspases -8 and -10 and the executioner caspases -3 and -7, and it has other substrates relevant to apoptosis induction including inhibitor of caspase-activated DNase and Bid [26,27]. The former mediates DNA fragmentation while the latter links perforin/granzyme to the mitochondrial pathway. Granzymes A and C are also implicated in apoptosis although their roles are less well characterized [28]. While perforin/granzymes is the primary mechanism for killing, CTL can also cause apoptosis by engaging Fas on target cells with FasL to trigger the extrinsic pathway [29].

2.2. Regulated necrosis

The field of programmed cell death has become increasingly complex since the discovery of apoptosis. Much recent interest has focused on pathways of regulated necrosis, which currently comprise necroptosis, pyroptosis, pyronecrosis, ETosis, cyclophilin D (CYPD)-dependent necrosis, parthanatos, and autophagic cell death [5]. Necroptosis is the best characterized pathway while pyroptosis, pyronecrosis and ETosis have been most closely identified in the context of infection. Necroptosis occurs when TNFR1 signaling is activated but caspase-8 is inhibited by drugs or virus-encoded anti-apoptotic proteins. This results in formation of a complex