Cell death as part of Innate Immunity: cause or consequence?

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Abstract

Regulated or programmed cell death play a critical role in development and tissue organization and function. In addition, it is intrinsically connected with immunity and host defense. Increasing cellular and molecular findings are changing the concept of cell death, revealing an expanding network of regulated cell death modalities and their biochemical programs. Likewise, recent evidences are demonstrating the interconnection between cell death pathways and how they are involved in different immune mechanisms. This work provides an overview of the main cell death programs and their implication in innate immunity not only as an immunogenic/inflammatory process, but also as an active defense strategy during immune response and at the same time, as a regulatory mechanism.

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ABSTRACT

Regulated or programmed cell death play a critical role in development and tissue organization and function. In addition, it is intrinsically connected with immunity and host defense. Increasing cellular and molecular findings are changing the concept of cell death, revealing an expanding network of regulated cell death modalities and their biochemical programs. Likewise, recent evidences are demonstrating the interconnection between cell death pathways and how they are involved in different immune mechanisms. This work provides an overview of the main cell death programs and their implication in innate immunity not only as an immunogenic/inflammatory process, but also as an active defense strategy during immune response and at the same time, as a regulatory mechanism.

keywords: programmed cell death, molecular pathways, apoptosis, regulated necrosis, innate immunity

Abbreviation List

PCD: Programmed cell death

RIPK: Receptor interacting protein kinases

MLKL: Mixed lineage kinase domain-like protein

TNF: Tumor necrosis factor

TNFR1: TNF receptor 1

FAS: FS-7 associated surface antigen

TRAIL: TNF-related apoptosis-inducing ligand

TRAILR: TRAIL receptor

ECM: Extracellular matrix

MPT: Mitochondrial permeability transition

CYPD: Cyclophilin D

PARP1: Poly-ADP ribose polymerase 1

GSH: Reduced glutathione

GPX4: Glutathione peroxidase 4

ETosis: Regulated necrosis with releasing of extracellular traps

GSDMD: Gasdermine D

NETosis : Regulated necrosis with Neutrophil Extracellular Traps

NOX4: NAPH oxidase 4

ERK: Extracellular signal-regulated kinase

MPO: Myeloperoxidase

NE: Neutrophil elastase

PAD4: peptidylarginine deiminase 4

ATG: autophagy-related genes

LC3: light chain 3

ROCK: Rho-associated coiled-coil containing protein kinases

IGF1R: insulin-like growth factor 1 receptor

NK: Natural killerlymphocytes

Bcl-2: B-cell lymphoma 2

Bid: BH3 interacting domain death agonist

PRR: Pathogen recognition receptor

NOD: Nucleotide-binding and oligomerization domain

IKK: IκB kinase

NΦ-κB: Nuclear factor-κB

IFN: Interferon

IPS-1: Interferon-βpromoter stimulator 1

HSP: Heat shock protein

DAP3: Death associated protein 3

DAMP: Danger associated molecular pattern

PAMP: Pathogen associated molecular patterns

RHIM: RIP homotypic interaction motif

DAI: DNA-dependent activator of IFN regulatory factors

TLR: Toll-like receptors

CTL: Cytotoxic T lymphocytes

PR3: Proteinase 3

CGD: chronic granulomatouse disease

3-MA: 3-methyladenine

LPS: lipopolysaccharide

ssRNA : single strand RNA

PKR: RNA-dependent protein kinase

CALCOCO 2 : calcium binding and coiled-coil domain containing protein 2

DC : Dendritic cells

INTRODUCTION

Cell death is a physiological event essential for homeostasis in multicellular organisms. However, it is also implicated in multiple pathological conditions such as neurodegenerative and ischemic disorders, cancer, auto-inflammatory and autoimmune diseases (Allam, Kumar, Darisipudi, & Anders, 2014; Godlewski & Kobylińska, 2016; Linkermann, Stockwell, Krautwald, & Anders, 2014). Consequently, cell death is tightly regulated by a strict biochemical program which depends on regulatory pathways and protein interaction networks. This programmed cell death (PCD) exhibits various types or modalities, according to the stimulus that triggers it and the molecular pathways that result activated (Galluzzi et al., 2018; Pasparakis & Vandenabeele, 2015). The multiple forms of PCD are in constant update by recent findings in this field, which are revealing the connections and molecular crosstalk between cell death programs, as well as their implication in a wide variety of biological processes (Yan, Elbadawi, & Efferth, 2020).

PCD is involved in several physiological functions such as organ development, tissue remodeling and epithelial cell renewal. It is also essential for cellular homeostasis and cell response to stress, acting as an intrinsic mechanism to prevent malignant transformation and cancer development (Godlewski & Kobylińska, 2016). In addition to its role in tissue maintenance, PCD is a crucial biological response for immunity and host defense. It is part of various innate and adaptive immune mechanisms/processes such as antiviral defense, killing of intracellular pathogens, inflammation, chemoattraction, lymphocyte selection and immune tolerance (Miao, Rajan, & Aderem, 2011; Sumpter Jr & Levine, 2010; Zhou & Fang, 2019). Increasing evidence is revealing novel functions of different cell death types in innate immunity, including the regulation and/or amplification of the inflammatory response, the modulation of cytokine production and release, as well as diverse microbe-killing strategies in specialized immune cells (Andrade & Darrah, 2013; Burgener & Schroder, 2020; H. Chen, Ning, & Jiang, 2017; Humphries, Yang, Wang, & Moynagh, 2015). This review will be focus in important findings that highlight the interconnection between various forms of PCD and their roles in innate immunity.

DIFFERENT TYPES OF CELL DEATH

Cell death is a multifactorial process that involves several molecular events and depends on multiple physiological conditions, thus it can be presented in a variety of fashions/modalities (D'Arcy, 2019; Gudipaty, Conner, Rosenblatt, & Montell, 2018). The different forms of cell death are driven by distinct molecular

pathways, although they share common regulatory factors and can be triggered by same stimuli depending on the cellular context. This section will present a general background of the main cell death programs currently identified.

Apoptosis and necroptosis

Apoptosis is the best characterized form of cell death, which is under molecular control and differs histologically and biochemically from necrosis, an unregulated or accidental cell death (reviewed in (Vanden Berghe, Kaiser, Bertrand, & Vandenabeele, 2015)). Until recently, apoptosis was considered the only form of PCD, however, the discovery of specific inhibitors of necrotic processes together with a systematic biochemical and genetic analysis, have redefined necrosis as a programmed and regulated cell death, also termed necroptosis (reviewed in (Gudipaty et al., 2018; Pasparakis & Vandenabeele, 2015; Vanden Berghe et al., 2015)).

Unlike accidental necrosis which is mainly caused by trauma, necroptosis is a biochemically controlled process activated by receptor interacting proteinkinases-1 and 3 (RIPK1 and RIPK3) and mediated by the mixed lineage kinase domain-like protein (MLKL) (Galluzzi, Kepp, & Kroemer, 2014; Linkermann et al., 2014; Vandenabeele, Galluzzi, Berghe, & Kroemer, 2010). The executioner mechanism of MLKL in necroptosis is not clear; nevertheless, two independent and non-exclusive models have been proposed to explain it. One of them is based on the recruitment of Ca^{2+} and Na^{+} ion channels by MLKL once inserted in plasma membrane, causing a massive osmotic imbalance that leads to membrane permeabilization and cell lysis, a distinctive feature of necroptotic cell death (Cai et al., 2014; X. Chen et al., 2014). The other model proposes a pore-forming mechanism by MLKL oligomerization at plasma membrane, which directly induces leaking of intracellular contents and cell lysis (Dondelinger et al., 2014; Su, Yang, Xu, Chen, & Yu, 2015).

Necroptosis could be induced by the proinflammatory cytokine tumor necrosis factor (TNF) under certain cellular conditions, through the signaling cascade of TNF receptor 1 (TNFR1) which lead to the activation of RIP kinases and formation of necrosome complex (reviewed in (Pasparakis & Vandenabeele, 2015)). TNFR1 also activates the apoptotic cascade by the recruitment of cytosolic complexes IIa and IIb, in a mechanism that is regulated by RIPK1 (Silke, Rickard, & Gerlic, 2015). Other membrane receptors that induce necroptosis include FAS (FS-7 associated surface antigen), TRAILR1 and TRAILR2 (TNF-related apoptosis-inducing ligand (TRAIL) receptor 1 and 2), which normally induce apoptosis through the activation of Caspase 8, but could recruit RIPK1 to initiate the formation of the necrosome under Caspase 8 blockade (Bertrand & Vandenabeele, 2011; Feoktistova et al., 2011; Humphries et al., 2015). Interestingly, RIPK1 seems to act as a molecular switch between apoptosis and necroptosis depending on the cellular conditions, a transition that is dynamically regulated by posttranslational modifications and protein expression (Feoktistova et al., 2011; Geng et al., 2017).

Furthermore, these types of PCD can be presented in specific variants, which are triggered by particular events and possess additional biological consequences. For example, anoikis is a special type of apoptosis which is induced by the loss of cell-cell/cell-extracellular matrix (ECM) contacts (Gilmore, 2005; Paoli, Giannoni, & Chiarugi, 2013). When cell is detached from neighboring cells or from the surrounding ECM, the disruption of cadherin or integrin interactions, respectively, triggers a caspase-dependent mechanism that ends in a PCD (Paoli et al., 2013). Selected cells can be also excluded from epithelial layers due to malignant transformation or overcrowding by a process called "extrusion", resulting in the activation of a biochemical program that eliminates extruded and detached cells by anoikis (Gudipaty et al., 2018). Likewise, regulated or programmed necrosis includes a variety cell death programs with a common cellular phenotype but different molecular pathways.

Other forms of programmed necrosis

Necroptosis is the best characterized form of programmed necrosis, but is not the only one. In general, all modes of regulated necrosis exhibit typical hallmarks like cellular rounding and swelling (known as oncosis), granulation at cytoplasmic level and plasma membrane rupture, however physiological and biochemical differences lead to a variety of sub-classes with particular mechanisms (reviewed in (Vanden Berghe et al., 2015)). These non-apoptotic cell death modalities include the mitochondrial permeability transition-

mediated regulated necrosis (MPT-mediated necrosis), parthanatos and ferroptosis. The first one, is induced by a mitochondrial pore composed of at least Cyclophilin D (CYPD) (the only component of the pore that has been identified so far), whereas parthanatosis caused by an excessive PARylation of intracellular proteins by Poly-ADP ribose polymerase 1 (PARP1) provoking a depletion of NAD^+ and ATP that leads to necrotic cell death (Galluzzi et al., 2018; Pasparakis & Vandenabeele, 2015; Vanden Berghe et al., 2015). On the other hand, ferroptosis involves an iron-dependent oxidative stress that is produced by a decrease in cysteine uptake (the oxidized form of cysteine), a deficit of GSH (reduced glutathione) and a depletion of the enzyme glutathione peroxidase 4 (GPX4) (Dixon et al., 2012; Pasparakis & Vandenabeele, 2015). In neurons, it has been reported a form of ferroptosis called oxytosis, which occurs as a result of glutamate toxicity by the blockade of the antiporter system X_c^-producing the deficit of cystine and the iron-dependent production of reactive oxygen species (Albrecht et al., 2010; Tan, Schubert, & Maher, 2001).

In addition, pyroptosis, pyronecrosis and regulated necrosis associated with the release of extracellular traps (termed as ETosis) are proinflammatory and microbial-induced forms of programmed necrosis which occur in specialized immune cells (Galluzzi et al., 2018; Vanden Berghe et al., 2015). Pyroptosis is triggered by canonical or non-canonical inflammasome stimulation, which induces activation of Caspase 1 or Caspase 11, respectively, while pyronecrosis has been described as a Caspase 1/Caspase 11-independent cell death that involves Cathepsin B release and lysosomal permeabilization (D'Arcy, 2019; Kepp, Galluzzi, Zitvogel, & Kroemer, 2010; Miao et al., 2011). The mechanism causing cell death has been better characterized in pyroptosis and is based in osmotic imbalance and cellular swelling like occurs in necroptosis, but the pore forming protein involved is Gasdermine D (GSDMD) instead of MLKL. GSDMD is proteolithicaly activated by active Caspase 1 upon inflammosome stimulation (X. Liu et al., 2016).

ETosis, also known as NETosis (from Neutrophil Extracellular Traps), occurs primary in neutrophils, but also in other innate immune cells, and is characterized by the release of chromatin structures with associated histones (called extracellular traps) that represents an efficient antimicrobial mechanism (Allam et al., 2014). The molecular events underlying this form of PCD have been characterized and it has been demonstrated that NAPH oxidase 4 (NOX4), a common enzyme in neutrophils, is a key component for the activation of this pathway. The hyperactivation of this enzyme in response to pathogens is mediated by the extracellular signal-regulated kinase (ERK), changing the ROS balance within the neutrophil and inducing Myeloperoxidase (MPO) and Neutrophil elastase (NE) activity, a downstream event that leads to chromatin condensation and massive permeabilization (including nucleus, granules and plasma membrane), interestingly through the pore forming protein GSDMD, a common feature with pyroptosis. It has been described that NE is implicated in GSDMD processing and activation, as well as histone cleavage, a modification that together with histone citrullination (mediated by peptidylarginine deiminase 4 or PAD4), facilitate DNA and chromatin rearrange. The final consequence is the extrusion of the extracellular trap with histones, proteases and granular proteins, resulting in the death of the neutrophil. Under certain conditions, the extrusion can occurs with neutrophil survival, a process termed as "vital NETosis". (Burgener & Schroder, 2020).

Autophagic cell death

Beyond apoptosis and regulated necrosis, autophagic cell death is receiving attention as an interesting third form of PCD. Autophagy is in fact, an adaptive mechanism essential for cell survival under unfavorable conditions such as starvation, extracellular or intracellular stress, high temperatures, overcrowding, hypoxia and antiproliferative stimuli (reviewed in (He & Klionsky, 2009)). During the autophagic mechanism, the cell orchestrates a complex response that includes different membrane rearrangements to form autophagosomes and ultimately autolysosomes, allowing the cell to digest and catabolize its own constituents to obtain energy and recycle intracellular molecules (He & Klionsky, 2009). However, this process can lead eventually to a form of cell death, in which the cell "eat itself", causing irreversible damage like loss of membrane and organelle integrity (Gudipaty et al., 2018; Tsujimoto & Shimizu, 2005). Therefore, autophagy is tightly regulated and depends on a sequence of specific intracellular events. This multistep process is mediated by a gene family termed as ATG (autophagy-related genes) and involves protein-protein interactions and posttranslational modifications, such as the lipidation of the microtubule-binding protein LC3 (light chain

3) with phosphatidylethanolamine and the degradation of p62 (reviewed in (Yang et al., 2015)). Autophagy is a classic pathway that has been studied for years, but more recently, autophagic cell death is getting attention as a defensive and regulatory mechanism rather than a deregulation of the process (D'Arcy, 2019; Yonekawa & Thorburn, 2013). According to this, autophagic cell death can acts in a similar way to apoptosis or necroptosis, representing another self-induced type of cell death in response to extracellular stressors or pathological stimuli.

Unconventional cell death programs

In addition to the classical forms of PCD and their emerging submodalities, other authors are reporting "particular" or "unconventional" ways in which cells die, involving distinct molecular events not fully understood yet. In line with this, phagoptosis and entosis have been described as specific forms of cell death which implicate the assimilation of a cell by other cell, a phenomenon that could lead to different outcomes (reviewed in (Gudipaty et al., 2018)). When a viable cell is phagocyted by other active live cell it falls undergo a process called phagoptosis, different from phagocytosis of apoptotic or dead cells by macrophages. It could be homotypic or heterotypic, if the fussed cells are of the same or different type, respectively. The molecular pathway has not been characterized in deep, but it requires the exposure of phosphatidylserine in cell surface and the loss of CD47, as occurs with the typical phagocytosis. The physiological importance of this process includes the turnover of erythrocytes and neutrophils and it has been described in some pathological conditions such as neuroinflammation (Gudipaty et al., 2018). On the other hand, entosis is the opposite process, when a viable cell invades another life cell, penetrating directly into the cytoplasm where is vacuolized, turning into an internalized or entosed cell. An entosed cell could be released resulting in survival, or killed dying by a lysosomal-dependent degradation that involves LC3 mediated vacuole targeting, recruitment and fusion. The entotic cell requires Rho-associated coiled-coil containing protein kinases (ROCK) activity and actino-myosin structures that facilitate the cell-into-cell penetration. This cell death modality is mainly implicated in epithelial tissue removal and embryo implantation (Galluzzi et al., 2018).

Some recent studies are also describing other vacuole-dependent forms of PCD termed as methuosis and paraptosis. The first one involves Ras hyper-activation and a massive accumulation of large single membrane vacuoles full of extracellular fluid which are derived from macropinosomes. The second variant is associated with cytoplasmic vacuolization as well, but these vacuoles are derived from expansion of endoplasmic reticulum and mitochondria. It could be driven by misfolded protein accumulation or Ca^{2+} overload and involves insulin-like growth factor 1 receptor (IGF1R) activation (Yan et al., 2020).

CELL DEATH AS PART OF INNATE IMMUNITY

As was described above, cell death is a dynamic phenomenon with specific purposes that is involved in a variety of biological functions. All forms of PCD represent biochemically controlled events with specific cellular functions, which are activated or downregulated depending on the physiological context. They can be also manipulated by pharmacological interventions, representing interesting targets to modulate different pathological conditions. The role of the main cell death modalities in particular biological functions, with special focus in innate immunity is discussed in this section.

Apoptosis and innate immunity

Apoptosis, the most classical and the best characterized form of PCD, represents a biological strategy that selectively eliminates infected cells to restrict the reproduction and propagation of viruses and intracellular pathogens (H. Chen et al., 2017). In this context, apoptosis seems to be stimulated by pathogen-cell interactions leading to Caspase 8 activation and initiation of the extrinsic apoptotic pathway (Yeretssian et al., 2011). This evolutionary mechanism is in fact highly effective and many intracellular pathogens have evolved to suppress apoptosis in a Caspase 8-dependent manner (Weng et al., 2014). In addition, apoptosis is an essential part of the cytotoxic mechanism displayed by innate immune cells like natural killer (NK) lymphocytes. It has been described that these effector cells induce apoptosis in microbe infected cells through specific serine proteases, called granzymes (Abbas, Lichtman, & Pillai, 2014). In the current model proposed to explain this process, perforins and granzymes released from cytoplasmic granules of NK cells are inter-

nalized by target cells during immune synapse, through endocytosis. Once in the endocytic compartment, membrane pore-forming proteins perforins facilitate the release of granzymes into the cytosol, where they induce apoptosis proteolyticaly activating executioner caspases (like Caspase 3) or members of the B-cell lymphoma 2 (Bcl-2) protein family, like Bid (BH3 interacting domain death agonist) (reviewed in (Prager & Watzl, 2019)). These lymphocytes also eliminate target cellsby apoptosis, expressing Fas and TRAIL receptors, which induce the apoptotic process by the intrinsic pathway via Caspase 8 activation (Prager & Watzl, 2019).

The molecular crosstalk between apoptosis and innate immunity is not limited to the executioner mechanisms and it also occurs during the regulation of cell response to infection. For example, the stimulator of the mitochondrial apoptotic pathway Bid is also involved in pathogen recognition receptor (PRR) signaling, inflammation and immunity. Bid seems to be recruited by nucleotide-binding and oligomerization domain (NOD) proteins after microbial DNA recognition, to form a complex with IκB kinase (IKK) and promote the activation of nuclear factor-κB (NF-κB) and extracellular signal-regulated kinase (ERK) pathways (Yeretssian et al., 2011). Through this dual role, Bid regulates how cells react to infection, either dying by an apoptotic process or surviving and displaying a pro-inflammatory and antimicrobial response (Yeretssian et al., 2011). In a similar way, TNF could stimulate pro-inflammatory and antimicrobial defensive pathways or induce apoptotic cell death, depending on the cellular conditions (Pasparakis & Vandenabeele, 2015). Additional examples of this interplay between apoptosis induction and innate immune response are related with the multifunctional role of other proteins that interconnect both processes, such as the Interferon-β promoter stimulator 1 (IPS-1) and heat shock proteins (HSPs). IPS-1 is involved in mitochondrial antiviral signaling and virus-induced Interferon-β (IFN-β) stimulation; however, it is also crucial to apoptosis and anoikis induction after cell detachment (Li et al., 2009). It has been experimentally demonstrated that IPS-1 once inserted in mitochondrial outer membrane, is able to recruit and activate Caspase 8 to induce anoikis by a distinct pathway that is independent of death receptor signaling or death associated protein 3 (DAP3) function (Li et al., 2009). On the other hand, HSPs which are apoptosis inhibitors and cytoprotective chaperones that promote cell survival to stress, can be translocated to plasma membrane or secreted to extracellular space to stimulate the immune system and enhance the immune response (Joly, Wettstein, Mignot, Ghiringhelli, & Garrido, 2010). HSP70 and HSP90 inhibit apoptosis interfering with death receptor signaling, restricting the mitochondrial permeabilization and cytochrome *c* release and preventing caspase activation, but in their extracellular form, they display a variety of immune functions including antigen presentation, cell recruitment and activation and a cytokine-like behavior (reviewed in (Joly et al., 2010)).

Apoptosis not only controls infected cells, but also transformed and malignant cells, an important function that is tightly related to immune surveillance and primary immune response to cancer (Su et al., 2015). The intrinsic apoptotic pathway is triggered by irreversible events such as irreparable DNA damage, disruption of cell division or cell cycle arrest, representing a crucial process to prevent genomic instability, increase in mutation rate and consequently, oncogenesis (Hanahan & Weinberg, 2011). Likewise, anoikis is a very effective mechanism to prevent metastasis, eliminating misplaced and detached cells (Zörnig, Hueber, Baum, & Evan, 2001). During tumor development, cancer cells must survive to pro-apoptotic conditions like hypoxia, growth factors deprivation, oxidative stress and metabolic deregulation, therefore apoptosis evasion is consider a hallmark of cancer (Hanahan & Weinberg, 2011; Su et al., 2015). Furthermore, as was described above, apoptotic cell death is crucial to NK cell-mediated cytotoxicity. This primary immune mechanism is critical to antitumor defense and innate immune response to cancer, particularly during carcinogenesis and early tumorigenesis (Pistritto, Trisciuoglio, Ceci, Garufi, & D'Orazi, 2016). Malignant cells have to avoid apoptosis in several stages to successfully become a tumor and colonize a distant organ. In this sense, this form of PCD acts as an intrinsic defense to suppress oncogenesis and neoplastic growth.

In addition to the above described functions, apoptosis also plays a key role in regulation and homeostatic balance after immune response. This is the biochemical program by which immune cells die while the infection is resolved, ensuring the decreasing of circling immune cells to the end of the response and avoiding an excessive damage to local tissues. Thus, the population of activated immune cells is regulated by the own infection, via dead receptors and Caspase 8 (Abbas et al., 2014; Feig & Peter, 2007). Apoptotic death

of neutrophils during bacterial, fungal or protozoal infection is a clear example of this scenario. Engulfed microorganisms accelerate neutrophil apoptosis ensuring a secure disposal of the phagocyted materials and ultimately the termination of the response, limiting the release of reactive oxygen species and at the same time recruiting and activating resident macrophages (Geering & Simon, 2011).

Regulated necrosis and innate immunity

Unlike apoptosis, which is considered a less immunogenic variant of PCD, all forms of regulated necrosis are highly inflammatory (Pasparakis & Vandenabeele, 2015; Vanden Berghe et al., 2015). This type of PCD involves the massive release of intracellular molecules which act as danger associated molecular patterns (DAMPs), activating the immune system and amplifying the inflammatory response (D'Arcy, 2019; Linkermann et al., 2014). The same process is triggered during innate immune response to microbes, inducing the release of pathogen associated molecular patterns (PAMPs) from infected cells, leading to immune cell recruitment and activation (Kaczmarek, Vandenabeele, & Krysko, 2013). This concept was introduced by certain studies that demonstrated that unlike apoptotic cells, necrotic cells are able to release specific molecules that activate immune cells like macrophages and antigen presenting cells. For example the research of Basu and colleges in 2000 who demonstrate that necrotic but no apoptotic E.G7 cells release HSPs such as hsp90, hsp70 and gp96 which can activate macrophages and dendritic cells through the stimulation of the NF-κB pathway. Although in this work they do not induce an specific necroptotic process (just a mimic or a regular necrosis), this was a first approach to the idea that during oncosis and non-apoptotic cell death the release of these intracellular factors is a crucial internal signaling to activate the immune response (Basu, Binder, Suto, Anderson, & Srivastava, 2000). This has been experimentally corroborated in the context of inflammatory necroptosis. For example, in spite of their pleiotropic role in apoptosis-necrosis transition, inflammosome activation and TNF signaling, the kinases RIPK1 and RIPK3 are actively involved in antiviral and antibacterial response in a necroptotic-dependent way (reviewed in (Humphries et al., 2015; Silke et al., 2015)). It has been demonstrated *in vitro* and *in vivo* that certain viral entities like cytomegalovirus or herpes simplex virus regulate the necroptotic response in murine infected cells through the interaction between viral encoded proteins and the RIP homotypic interaction motif (RHIM) domains of RIPK1 and RIPK3 (Huang et al., 2015; Upton, Kaiser, & Mocarski, 2008, 2010). This has also been observed in human infected cells (Omoto et al., 2015). However, necroptosis can be also manipulated as a microbial strategy to impair immune function. In line with this, intracellular bacteria such as *Salmonella enterica* are also strong inducers of RIPK3-dependent necroptosis in infected macrophages (Robinson et al., 2012). The same mechanism is used by other pathogenic entities, which induce necroptosis in immune cells to interfere with its defensive function and amplify the inflammatory loop (Weinlich, Oberst, Beere, & Green, 2017). In spite of that, necroptosis is still an efficient mechanism to eliminate and expose intracellular pathogens. Some bacteria have even evolved to suppress necroptosis as an infective mechanism. That is the case of *Porphyromonas gingivalis* , which is able to cleave RIPK1 in human infected endothelial cells via a lysine-specific (Kgp) protease (Madrigal, Barth, Papadopoulos, & Genco, 2012). The key role of RIP kinases and necroptosis in the maintenance of an immunocompetent status has been confirmed *in vivo* , in RIPK3-knockout mice which are more susceptible to viral and bacterial infections (Jorgensen, Rayamajhi, & Miao, 2017; Kaiser, Upton, & Mocarski, 2013). Moreover, the role of RIP kinases in innate immunity is beyond necroptosis induction. RIPK1 and RIPK3 are key regulators of cell final destination during an infection or immune challenge, but they can also participate in signal transduction for the downstream activation of NF-κB acting as adaptor proteins in the signaling cascade of nucleic acid sensing proteins like the DNA-dependent activator of IFN regulatory factors (DAI) or Toll-like receptors such as TLR3 and TLR4 (Humphries et al., 2015).

Not only necroptosis but other forms of regulated necrosis are also implicated in particular and interesting mechanisms of innate immunity. One of the clearest examples is the form of PCD called Pyroptosis/Pyronecrosis, which receives this denomination due to its final and distinctive molecular event: the releasing of pyrogenic cytokines like IL-1β and IL-1α (Κεππ ετ αλ., 2010). This special form of PCD occurs in different cell types but primary in immune cells, mainly macrophages, as a direct response to pathogens leading to the Caspase 1-dependent processing and release of IL-1β, a potent inflammation mediator and IL18, which induces Interferon-γ (IFN-γ) production in TH1 cells, NK cells and cytotoxic T lymphocytes

(CTLs), and also promotes TH2 cell development (Dinarello, 2009; B. Liu et al., 2004; Miao et al., 2011). However, besides of its function in cytokine maturation and release, pyroptosis itself seems to be an efficient mechanism to eliminate and release intracellular bacteria from infected macrophages, exposing them to neutrophil-mediated clearance trough a molecular mechanism that relies on Caspase 1 activity. This has been demonstrated *in vivo* for *Salmonella typhimurium, Legionlla pneumophila* and *Burkholderia thailandensis* in *Casp1* knockout mice (Miao et al., 2011). This form of programed necrosis has been recently recognized by its role in the immune response against bacterial infections of the gastrointestinal tract and even in the control of transformed cells in the context of gastrointestinal cancer (Zhou & Fang, 2019).

On the other hand, NETosis is another important example of how regulated necrosis actively mediates microbe killing and immune enhancing. This cell death modality is known to be induced in specialized immune cells like neutrophils, leading to the release of the so called "extracellular traps", complex structures composed of chromatin fragments and histones which opsonize and enclose circling pathogens, limiting their spreading within the body and facilitating their clearance by phagocytocis (Allam et al., 2014; Andrade & Darrah, 2013). These extracellular traps also contain antimicrobial proteins such as, MPO, NE, Proteinase 3 (PR3), Cathepsin G, Lysozyme and ⍺-defensins representing a non-phagocytic mode of neutralize and degrade invading pathogens (Branzk & Papayannopoulos, 2013; Urban et al., 2009) and are able to stimulate the production of Interferon-α (IFN-α), a strong antiviral cytokine (Garcia-Romo et al., 2011). In the past 10 years, this form of PCD has been extensively studied to elucidate the sequence of its molecular events and its role in immune response, demonstrating that is actually one of the most specialized immune mechanism, which can be activated depending on microbe size and location, and as a strategy to control pathogens which are able to escape from neutrophil phagocytosis (reviewed in (Burgener & Schroder, 2020)). The effectiveness of this antimicrobial defense is evident in some pathogens such as Group A *Streptococcus* and *Staphylococcus aureus* , that produce nucleases and DNA binding proteins to impair NET formation and block NETosis as an active evasion mechanism (Storisteanu et al., 2017). In the same way, it has been reported that chronic granulomatouse disease (CGD) patients are more susceptible to *Aspergillus nidulans* growth, due to their deficiency in NADPH oxidase function and consequently in NET formation (Bianchi et al., 2009). Invasive aspergillosis is a leading cause of death in CGD patients, suggesting the importance of NETosis in pathogen control and immunocompetent status.

Autophagic cell death and innate immunity

Different experimental evidences have revealed the presence of autophagosomes and the rearrangement of the cytoskeleton in dying cells, but wherever this is a strategy of cell survival rather than an active death mechanism, has been a controversy. Conclusive data, using apoptosis inhibitors and Bax/Bak-deficient mouse embryonic fibroblasts have showed that autophagic cell death is an alternative to apoptosis in which cell dies in response to chemical stressors or cytotoxic substances like staurosporine or etoposide, through a process that involve the formation of multiple vesicular organelles and can be reversed using classical autophagy inhibitors such as 3-methyladenine(3-MA) or silencing ATG genes (reviewed in (Tsujimoto & Shimizu, 2005)). It seems that like regulated necrosis, autophagic death is activated under certain stimulus including infection when apoptosis is blocked or inhibited, playing a protective role in host defense. In line with this, different *in vivo* studies have demonstrated the importance of the*ATG* genes against bacterial and viral infections such as*Listeria monocytogenes, Toxoplasma gondii* , herpes simplex virus 1 and Sindbis virus in mice (Orvedahl et al., 2007; Orvedahl et al., 2010; Zhao et al., 2008).

As occurs with apoptosis and regulated necrosis, autophagic cell death can be activated by PRRs as result of direct contact with pathogens, inducing an alternative defense that helps cells to deal with viruses or intracellular microorganisms, a key element of innate immune response. The activation of the autophagic death by TLRs such as TLR1/2, TLR3, TLR4 and TLR7 has been corroborated in mice-derived macrophages and dendritic cells, in response to agonists like bacterial lipopolysaccharide (LPS) or under *Mycobacterium tuberculosis*infection. In the same way, peptidoglycan has demonstrated to induce autophagy through NOD proteins, mainly Nod1 and Nod2 in murine myeloid and epithelial cells (reviewed in (Sumpter Jr & Levine, 2010)). Interestingly, muramyldipeptide a peptidoglycan derived product, also activates autophagy in human

dendritic cells, in a process that requires *NOD2, RIP2* , and the *ATG* genes,*ATG5, ATG7* , and *ATG16L1* , increasing MHC Class II antigen expression and CD4+ T lymphocytes proliferation (Cooney et al., 2010). Not only bacterial but viral infections also induce an autophagic defensive response in host cells. Viral single strand RNAs (ssRNAs) are recognized by TLR7 and NOD2 stimulating antiviral defense in RAW 264.7 macrophages and type I IFN production in dendritic cells. The ssRNAs can additionally activate other intracellular sensors like RNA-dependent protein kinase (PKR) which activate autophagy via Beclin-1 in mouse embryonic fibroblasts and primary neurons infected with herpes simplex virus 1 (Gu, Wang, & Yang, 2014).

The process of autophagy by itself is intrinsically connected to innate immune clearance of intracellular pathogens. Thus, when a pathogen interacts with a host cell it triggers an autophagic process that could lead to its own phagocytosis or eventually to the death of the infected cell, exposing this microbe to other effector cells of the immune system. Experimental results with intracellular *Salmonella* , enteropathogenic *Echerichia coli* , *Streptococcus pyogenes*and *L. monocytogenes* show that engulfed bacteria are targeted to autophagosomes and subsequently to autolysosomes by a coat of ubiquitin that interacts with the cytoskeleton protein LC3. This was corroborated by colocalization and confocal microscopy studies, together with the use of *Atg5* or *p62* deficient cells and genetically modified microbial strains insensitive to ubiquitin recognition (Sumpter Jr & Levine, 2010). In the context of infection, different autophagic signalling proteins such as p62, CALCOCO 2 (calcium binding and coiled-coil domain containing protein 2) and optineurin can act as adaptor proteins in the inflammatory pathway ultimately activating NF-ϰB and cytokine secretion. Additionally, p62 is also involved in the recruitment of cytosolic proteins such as ubiquitin or ribosomal proteins to autolysosomes, to be converted by internal proteolysis in antimicrobial peptides, highly effective to intracellular pathogens like *M. tuberculosis*(reviewed in (Deretic, 2012).

The processes of autophagy and autophagic cell death have been also revised due to its controversial role in cancer. Some studies reveal that autophagy actually contribute with survival, metabolic reprogramming and tumor development. Interestingly, the same process can lead to the elimination of cancer cells by autophagic cell death (Yang et al., 2015). Autophagy is also required for immune cell activation, antigen presentation, thymus selection and cytokine release in different cell types such as NK cells, Dendritic cells (DC) and T lymphocytes, but represents a "double edge sword" that can also regulate these cell populations by autophagic cell death. Current research is focus on the understanding of this dual role and its potential application in cancer immunotherapy (Jiang et al., 2019).

CONCLUSIONS AND PERSPECTIVES

Programmed cell death (PCD) in its different modes of presentation and acting through different pathways has proved to be more that the final fate of a damaged or a senescent cell. This is a complex process which can be induced, sustained, adjusted and even reversed according to the cell type, the intra and extracellular conditions, the stimulus that triggers it and the interactions with other surrounding cells. Several experimental data have demonstrated that PCD is actively involved in a variety of biological functions but especially in innate immunity and host defense. As it has been discussed throughout this review, PCD is induced by pathogen presence and is part of the immune mechanisms involved in their detection and elimination. Different types of pathogens are able to induce different cell death modalities, indicating that is a specialized process rather than just a physiological consequence of microbe invasion to host cells. PCD is no longer seen as the result of an inflammatory process but also as fine-tuned mechanism that can induce, amplify or modulate this inflammation, either during physiological or pathological conditions, including infection and malignant transformation. The expanding network of cell death programs is revealing novel and complex variants or PCD and their key role in innate immunity and immune cell homeostasis. The existence of multiple forms for a cell to die and their involvement in different immune mechanisms offers interesting ways to selectively modulate and enhance immune response to infections, chronic inflammation and cancer, through pharmacological interventions and immunotherapy.

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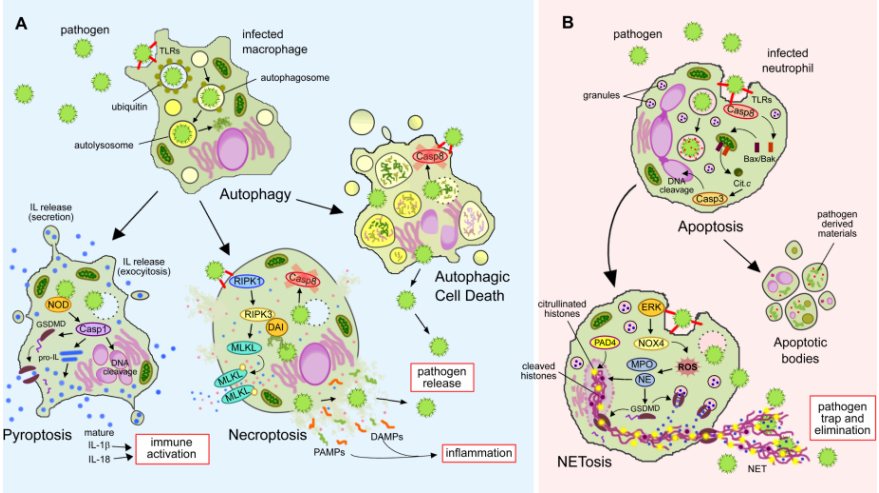

FIGURES

Figure 1. Schematic view of the interplay between different cell death modalities in the context of infection. During immune challenge, in infected macrophages (A) an autophagic process can be triggered by pathogen contact and the phagocyted microorganisms are targeted by an ubiquitin coat to autophagosomes and then to autolysosomes to be eliminated by acidic and enzymatic degradation. If microbes are persistent or escape from vacuolar compartments, under apoptosis blockade (for example by Caspase 8 inactivation) alternative cell death programs can be activated, releasing the pathogen to be neutralized by other immune cells, enhancing inflammation and activating the immune system. Thus, the cell can go undergo autophagic cell death, necroptosis induced by TLRs under Caspase 8 inhibition, through the RIPK1-RIPK3-MLKL pathway or by Microbial DNA through DAI-RIPK3 interactions, or Caspase 1-dependent pyroptosis triggered by NOD receptors. On the other hand, in infected neutrophils (B) pathogen contact triggers an apoptotic process that ensures the safe disposal of the microbial components and toxins after its degradation and at the same time regulates neutrophil population during immune response. Alternatively, persistent pathogens can also activate NETosis, through the ERK-NOX4 pathway, Massive permeabilization and releasing of granules content cause neutrophil death, but the released extracellular traps enclose and destroy invader pathogens. TLRs: Toll-like receptors; RIPK: Receptor interacting protein kinases; MLKL: Mixed lineage kinase domain-like protein; DAI: DNA-dependent activator of IFN regulatory factors; PAMPs: Pathogen associated molecular patterns; DAMPs: Danger associated molecular patterns; NOD: Nucleotide-binding and oligomerization domain; GSDMD: Gasdermine D; IL: Interleukine; ERK: Extracellular signal-regulated kinase; NOX4: NAPH oxidase 4; ROS: Reactive oxigen species; MPO: Myeloperoxidase; NE: Neutrophil elastase; PAD4: peptidylarginine deiminase 4; NET: Neutrophil extracellular trap.