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Opinion

Pyroptosis: Gasdermin-Mediated Programmed Necrotic Cell Death

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Pyroptosis was long regarded as caspase-1-mediated monocyte death in response to certain bacterial insults. Caspase-1 is activated upon various infectious and immunological challenges through different inflammasomes. The discovery of caspase-11/4/5 function in sensing intracellular lipopolysaccharide expands the spectrum of pyroptosis mediators and also reveals that pyroptosis is not cell type specific. Recent studies identified the pyroptosis executioner, gasdermin D (GSDMD), a substrate of both caspase-1 and caspase-11/4/5. GSDMD represents a large gasdermin family bearing a novel membrane pore-forming activity. Thus, pyroptosis is redefined as gasdermin-mediated programmed necrosis. Gasdermins are associated with various genetic diseases, but their cellular function and mechanism of activation (except for GSDMD) are unknown. The gasdermin family suggests a new area of research on pyroptosis function in immunity, disease, and beyond.

Programmed Cell Death and Pyroptosis

Apoptosis, the prototype of programmed cell death, is essential for multicellular organism development. Apoptosis features activation of the caspase family of cysteine proteases; the initiator caspase receives extrinsic or intrinsic apoptotic cues and then activates the executioner caspases to initiate the death program. In apoptosis, cells shrink and are fragmented into apoptotic bodies that are usually engulfed by surrounding macrophages, leading to the noninflammatory nature of the cell death. Inhibiting apoptotic caspase (caspase-8) in the presence of the proapoptotic stimulus triggers necroptosis, a form of programmed necrotic cell death, through the receptor-interacting serine/threonine-protein kinase 1 (RIPK1)–RIPK3-mixed lineage kinase domain-like (MLKL) axis [1]. RIPK3-phosphorylated MLKL moves to the membrane to execute membrane disruption, causing cell swelling and lysis [2–5].

Pyroptosis, the other form of programmed necrosis, is emerging as a general innate immune effector mechanism in vertebrates [6]. Innate immunity relies on pattern recognition receptors (PRRs) to detect conserved microbial products or endogenous dangers. Besides inducing cytokine transcription, activation of PRRs, particularly those in the cytosol, can also induce pyroptosis to stimulate inflammatory responses. The immune defense function of pyroptosis is strengthened by its disruption of the pathogen replication niche and direct killing of intracellular bacteria through pore-induced intracellular traps [7,8]. For a long time, pyroptosis has been referred to as caspase-1-mediated monocyte death [9]. Caspase-1 [originally named as ICE for interleukin (IL)-converting enzyme], belonging to the inflammatory caspase group, was the first caspase identified via its activity of processing pro-IL-1β into mature IL-1β [10,11]. This proinflammatory nature distinguishes pyroptosis from apoptosis despite the dependency on

Trends

The necrotic nature of pyroptosis was not well appreciated for decades and it was misregarded as a special type of apoptosis in monocytes due to the involvement of a caspase (caspase-1).

Characterization of inflammasomes establishes caspase-1-mediated pyroptosis as a general innate immune effector mechanism.

Caspase-4, 5, and 11, expressed also in nonmonocytic cells, induce pyroptosis upon recognition of intracellular lipopolysaccharide. The role of caspase-11 in endotoxic shock emphasizes the physiological importance of pyroptosis.

Both caspase-1 and caspase-11/4/5 cleave gasdermin D (GSDMD), a gasdermin-family member, to release its gasdermin-N domain that perforates the plasma membrane to induce cell swelling and osmotic lysis.

Nearly all gasdermins share the pore-forming and pyroptotic activity of GSDMD. Several gasdermins are associated with genetic diseases but their function and activation mechanism are unknown.

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Box 1. Early Observation of Pyroptosis and Caspase-1-Mediated Monocyte Death

The earliest observation of what is now known to be pyroptotic cell death can be traced to a 1986 report, in which Arthur Friedlander showed that treating primary mouse macrophages with anthrax lethal toxin (LT) could induce robust cell death with rapid release of cellular contents [66]. Efforts toward investigating LT-induced cytokine release such as interleukin-1β (IL-1β)/IL-18 then revealed caspase-1 activation in LT-treated macrophages [67]. LT-induced cell death depends on the genetic background of the macrophage. Meanwhile, apoptotic cell death (resulting from LT cleavage of host mitogen-activated protein kinases) was also reported in certain LT-treated macrophages. This caused confusions in understanding LT-induced death before the identification of *Nlrp1b* as the determinant for macrophage susceptibility to LT-induced caspase-1-dependent death [68]. The other line of early pyroptosis research originates from the observation that *Shigella flexneri* infection could cause severe death in mouse macrophages, which was previously misclassified as apoptosis [69]. Subsequently, similar 'apoptosis' was noted in macrophages infected with *Salmonella typhimurium*, an enteric bacterium closely related to *S. flexneri*. This caspase-3-independent and Bcl-2-resistant 'apoptosis' was found to also require caspase-1 via a mistaken route [70].

LT and *S. flexneri*-induced macrophage death features IL-1β secretion [67,71], which is in contrast to the immunologically silent nature of apoptosis. However, the prevailing concept in 1990s – that all caspases function in apoptosis – prevented the realization that LT- and *S. flexneri*/*S. typhimurium*-induced macrophage deaths are indeed necrotic, despite some neglected voices arguing for the latter [72,73]. To distinguish this proinflammatory 'apoptosis' from canonical apoptosis, Brad Cookson and his coworkers invented a new term, 'pyroptosis', in which the Greek roots 'pyro' and 'ptosis' mean heat/fever and falling off, respectively [74]. DNA cleavage and poly(ADP-ribose) polymerase activation, characteristics of apoptosis, are observed in *S. typhimurium*-infected macrophages but are not required for cell death [75]. Instead, caspase-1-dependent osmotic lysis following cell swelling was proposed to be the cause of pyroptotic cell death [76]. Because of the complications of bacterial infection and the involvement of caspase activation, pyroptosis is not recognized as a form of necrosis by the cell death community but classified as an atypical cell death with mixed apoptotic and necrotic features [77]. Given that *S. flexneri*/*S. typhimurium* and LT induce caspase-1 activation only in macrophage cells, pyroptosis was classified as an atypical and monocyte-specific proinflammatory cell death then.

a caspase. Pyroptosis is lytic, featuring cell swelling and large bubbles blowing from the plasma membrane [6], but the necrotic nature only became generally accepted in recent years. Exciting advances in the past few years have revolutionized the concept of pyroptosis, which is the main subject of this opinion article. For completeness, we begin with the historic perspective of pyroptotic cell death (Box 1).

Caspase-1 Is Activated by Various Inflammasome Complexes in Innate Immunity

In 2002, Jürg Tschopp [12] proposed the inflammasome complex as a molecular platform for caspase-1 activation, according to the genetic understanding of IL-1β-associated autoinflammatory diseases and biochemical knowledge of the death-domain superfamily of proteins. Assembly of the oligomeric inflammasome complex, in response to infection or a certain immunological challenge, is mediated by a PRR and a downstream adaptor such as ASC or NLRC4; the PRR detects the particular stimulus and signals to the adaptor that binds to caspase-1 and oligomerization of the PRRs brings multiple caspase-1 molecules into proximity for activation. Extensive studies in the past decade have recorded a handful of inflammasomes that detect specific microbial challenges and endogenous dangers [13] (Figure 1). Among the best characterized ones are the AIM2/ASC inflammasome, which recognizes cytosolic double-stranded DNA with AIM2 as the DNA-binding receptor; the NAIP/NLRC4 inflammasomes, which directly recognize bacterial flagellin and type III secretion apparatus with NAIP and NLRC4 as the receptor and signaling amplifier, respectively; the NLRP3/ASC inflammasome, which is activated by various membrane-damaging agents; the Pyrin/ASC inflammasome, which indirectly senses inactivating modifications of host Rho GTPases by bacterial toxins; and the NLRP1 inflammasome, which detects anthrax lethal toxin activity (as described earlier) or *Toxoplasma gondii* infection. Activation of the inflammasome pathways appears to feature much more diversified biochemical mechanisms than originally proposed [12,14]. The identification of different inflammasomes has established that caspase-1 and caspase-1-mediated pyroptosis is of paramount importance as a general innate immune defense mechanism.

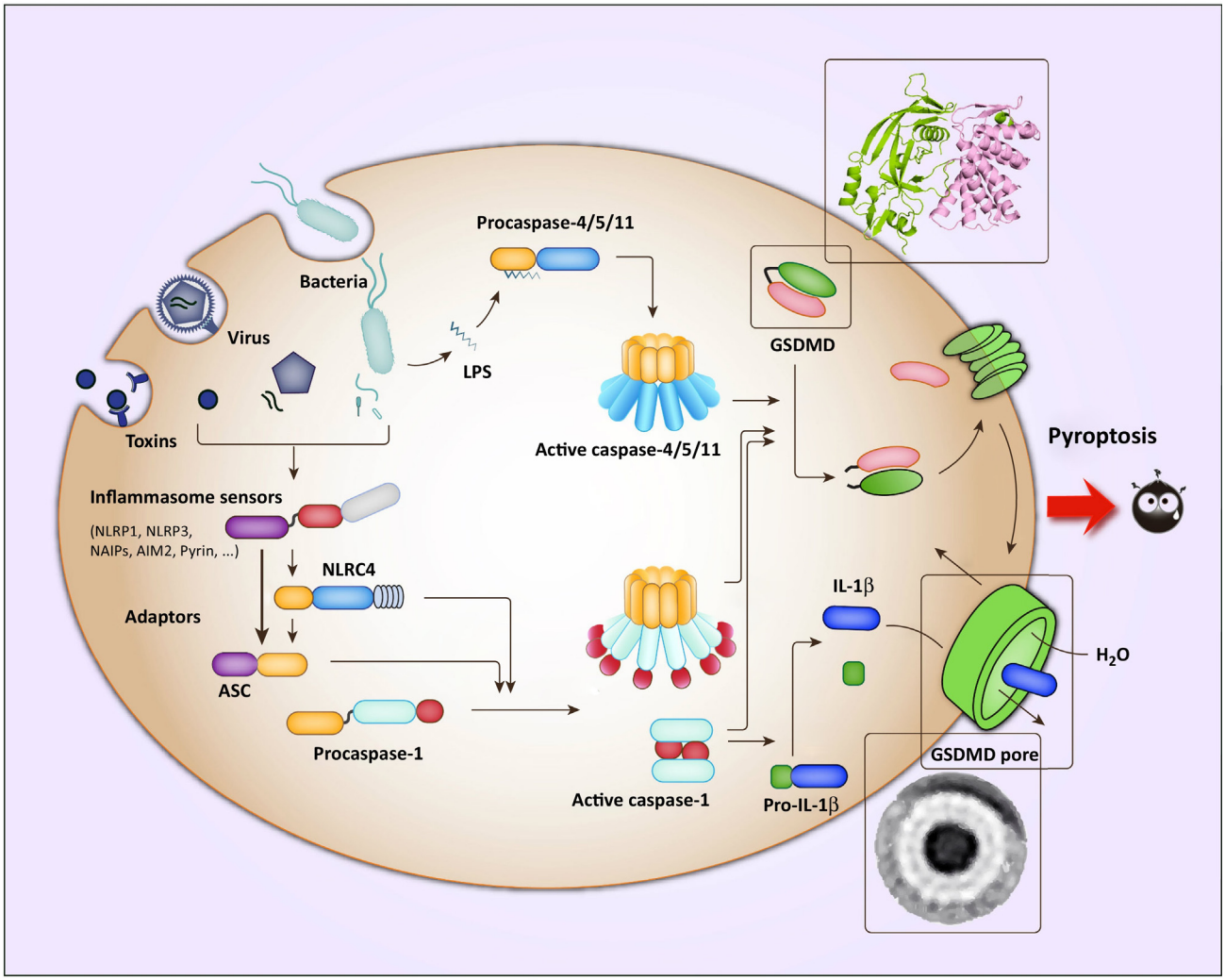

Figure 1. GSDMD Executes Inflammatory Caspases-Induced Pyroptosis by Generating Pores on the Plasma Membrane. The canonical inflammasome sensors detect diverse microbial signals and activate caspase-1 through the ASC or NLRC4 adaptor. Caspase-4, 5, and 11 are activated by direct binding to LPS. Active caspase-1 and caspase-11/4/5 cleave GSDMD within the linker between its N-terminal gasdermin-N (green) and C-terminal gasdermin-C (pink) domains to break the autoinhibitory interactions between the two domains. The released gasdermin-N domain binds to phosphoinositides in the plasma membrane and oligomerizes to generate membrane pores of about 12–14 nm in inner diameter. The formation of the pores disrupts the osmotic potential, resulting in cell swelling and eventual lysis. The pores can also serve as a gate for extracellular release of mature IL-1β. The crystal structure of GSDMA3 and the negative electron microscopy picture of GSDMD pores shown on the upper and lower right, respectively, are experimental data reported in and reprinted with permission from [35]. GSDMD, gasdermin D; IL, interleukin; LPS, lipopolysaccharide.

Caspase-11/4/5 Also Triggers Pyroptosis upon Recognition of Cytosolic Lipopolysaccharide

Casp1 knockout mice are highly resistant to lethal injection of lipopolysaccharide (LPS) [15]. However, the characterization of various inflammasomes in the past 10 years has only revealed a role for LPS in the transcriptional priming of certain inflammasome components. Intriguingly, mice lacking *Casp11*, another inflammatory caspase encoded adjacent to *Casp1* on the chromosome, also resist lethal LPS injection. Caspase-11 expression is strongly induced by LPS stimulation [16,17]. A 2000 study noted that *Casp1*$^{-/-}$mice, generated through the conventional 129S2/SvPas mice-derived embryonic stem cell targeting, do not express caspase-11

even after LPS stimulation [18]. These observations urge reexamination of the endotoxic resistance observed in the original *Casp1*$^{-/-}$mice. Recently, a 5-nt deletion was identified in *Casp11* of strain 129 mice (which produce a truncated nonfunctional caspase-11), and it is the loss of *Casp11* but not *Casp1* that could protect mice from endotoxic shock [19]. Subsequent studies found that caspase-11 triggers mouse macrophage death in response to various Gram-negative infections [19–21]. This cell death is pyroptosis as it morphologically resembles that induced by caspase-1. Consistent with LPS being the major component of the Gram-negative bacterial cell wall, cytoplasmic LPS was identified to be the bacterial signal responsible for caspase-11-mediated pyroptosis [22–24]. Further, the role of Toll-like receptor 4 in determining endotoxic shock turns out to be limited to transcriptional priming of caspase-11 rather than the presumed cytokine storm.

How is cytosolic LPS sensed in activating caspase-11-mediated pyroptosis? Guided by the model of caspase-1 activation, a 'noncanonical inflammasome' was proposed, in which a cytosolic PRR, most likely a caspase activation and recruitment domain (CARD)-containing protein, recognizes LPS and then oligomerizes to form a multiprotein complex for caspase-11 activation [19]. Unexpected from this plausible hypothesis, caspase-11 itself indeed directly recognizes LPS via specific and high-affinity binding to the lipid A moiety in LPS [25]. The binding triggers caspase-11 oligomerization and consequently activation of its proteolytic activity. Truncation and chimera studies reveal that the CARD in caspase-11 is responsible and sufficient for binding to lipid A. Caspase-4 and caspase-5 in humans appear to have the same function and are also activated by direct LPS binding. Thus, caspase-11 as well as caspase-4/5 functions as both PRRs and pyroptosis inducers in innate immunity (Figure 1).

Caspase-4 is expressed and functions not only in monocytes but also in various non-monocytic cells including epithelial cells and keratinocytes [25]. Recent studies further reveal that both caspase-11 activation and caspase-1 activation play important roles in intestinal epithelial defense against bacterial infections [26,27]. Thus, pyroptosis is not limited to monocytes as originally thought and can occur in multiple cell types. Caspase-11/4/5 do not process pro-IL-1β and IL-18; however, caspase-11 activation does induce a low level of IL-1β secretion in an NLRP3 inflammasome-dependent manner. This is an indirect result of membrane damage in pyroptotic cells. The NLRP3 inflammasome is absent in many human nonmonocytic cells that harbor a functional caspase-4-mediated LPS sensing pathway [25]. Thus, pyroptosis is the dominant response upon caspase-11/4/5 recognition of cytosolic LPS, and the recognition can induce NLRP3-mediated IL-1β/18 secretion only in certain subsets of cells.

Caspase-1 and Caspase-11/4/5 Cleave Gasdermin D to Trigger Pyroptosis

How does inflammatory caspase activation trigger pyroptosis? The question did not receive much attention for more than 20 years. Previous apoptosis research indicates that there is a high probability that the caspase may cleave multiple substrates to drive pyroptosis. However, two independent studies now show that it is not the case and identify a single gasdermin D (GSDMD) protein as the key pyroptosis substrate of inflammatory caspases [28,29] (Figure 1). One group performed *N*-ethyl-*N*-nitrosourea mutagenesis screening for mutant mice whose bone marrow macrophages showed impaired IL-1β secretion upon cytosolic LPS stimulation. The other study employed the clustered regularly interspaced palindromic repeat (CRISPR)/Cas9 genome-editing technology and carried out genome-wide screening of caspase-1 and caspase-11-mediated pyroptosis in mouse macrophages, both of which also hit *Gsdmd*. The name GSDMD comes from a homologous mouse gene (now known to be *Gsdma*) that was found to be highly expressed in gastrointestinal tissue and skin [30]. *Gsdmd* is widely expressed in different tissues and cell types, with high levels of expression in the gastrointestinal epithelia [31], which supports the notion that pyroptosis is not limited to macrophages.

GSDMD is highly conserved in mammals, but its function is unknown [32]. GSDMD contains about 480 amino acids divided into two domains, the N-terminal gasdermin-N domain and the C-terminal gasdermin-C domain, which are linked by a long loop. Activated caspase-1 and caspase-11 efficiently cleave GSDMD at an aspartate site within the linking loop. The same cleavage was also observed with LPS-activated caspase-4/5, and blocking the cleavage renders cells completely resistant to cytosolic LPS [29]. This is also true for caspase-1-mediated pyroptosis upon activation of any of the known inflammasomes; however, GSDMD-deficient cells may still die eventually, likely due to caspase-1 cleavage of caspase-3/7 [29,33,34]. GSDMD is insensitive to apoptotic caspases. Engineering the cleavage site in GSDMD into the caspase-3-recognition site switches tumor necrosis factor-α-induced apoptosis into pyroptosis, suggesting that interdomain cleavage of GSDMD is sufficient for pyroptosis. This phenomenon also demonstrates that pyroptosis has faster kinetics than apoptosis. Furthermore, *Gsdmd* deficiency blocks IL-1β secretion without affecting its processing by caspase-1 [28,29]. Thus, pyroptosis may mediate the noncanonical secretion of some inflammatory cytokines.

The Gasdermin-N Domain of GSDMD Forms Membrane Pores to Trigger Pyroptosis

Expression of the gasdermin-N domain of GSDMD in mammalian cells is sufficient to induce pyroptosis [28,29,35]. Full-length GSDMD is inactive due to inhibitory binding to gasdermin-N by its gasdermin-C domain. The gasdermin-N domain is also extremely toxic to bacteria [35], raising a hypothesis that it may directly target the membrane and lyse it. Supporting this prediction, recombinant gasdermin-N of GSDMD shows robust and specific binding to phosphoinositides and cardiolipin [35,36], two lipids known to be present in the mammalian cell plasma membrane and bacterial inner membrane, respectively. Consistently, the gasdermin-N domain was found to associate with membranes, including the plasma membrane [35–38]. Gasdermin-N does not bind to unphosphorylated phosphatidylinositol, indicating recognition of the negatively charged head group. Lipid binding induces oligomerization of gasdermin-N *in vitro* and during pyroptosis [35,37,38]. The oligomerized gasdermin-N then causes severe leakage of liposomes or lysis of biomembranes. All these properties of the gasdermin-N domain echo those of bacterial pore-forming toxins. The gasdermin-N domain of GSDMD can form extensive pores on phosphoinositide- or cardiolipin-containing liposomes or on liposomes made of natural polar lipid mixtures [35–38]. The pores mostly have an inner diameter of 12–14 nm with approximately 16 symmetric protomers [35]. Thus, GSDMD is a pore-forming protein that normally exists in the autoinhibited state. Different from known pore-forming proteins, GSDMD can only cause cell lysis from within mammalian cells due to the asymmetric distribution of phosphoinositides on the plasma membrane [35,36]. The formation of membrane GSDMD pores disrupts the osmotic potential, causing cell swelling and eventual lysis. The MLKL protein has also been suggested to be directly responsible for membrane lysis in necroptosis. However, a recent study has suggested some morphological differences between pyroptosis and necroptosis [39], which are likely attributed to the different biochemical activities in GSDMD and MLKL. Moreover, it has also been proposed that activated GSDMD may form pores on bacterial membranes to kill the intracellular bacteria [36], but it remains to be determined how GSDMD protein could pass through the thick cell wall to gain access to bacterial inner membrane. Further *in vivo* functional evidence is required to support the formation of GSDMD pores on bacterial membrane. Despite this, identification of the pore-forming activity in GSDMD has uncovered the biochemical basis of pyroptosis (Figure 1).

The Gasdermin Family Shares the Propyroptotic Activity and Autoinhibited Structure

GSDMD belongs to a gasdermin family that shares about 45% sequence homology, with the gasdermin-N domain being the most conserved region. Humans harbor GSDMA, GSDMB,

Table 1. Summary of the Gasdermin Family Properties and Functions

Gene name	Chromosomal location	Domain[a]	Expression pattern[b]	Pore-forming activity	Activation mechanism	Biological function	Disease
GSDMA (*Gsdma1–3*)	17q21.1 (11D)	N + C	Esophagus, stomach, skin	✓	?	?	Alopecia
GSDMB	17q12	N + C	Esophagus, stomach, liver, colon, intestine, T cells	✓	?	?	Childhood asthma
GSDMC (*Gsdmc1–4*)	8q24.21 (15D1)	N + C	Esophagus, stomach, trachea, spleen, skin, intestine	✓	?	?	Not known
GSDMD (*Gsdmd*)	8q24.3 (15D3–E1)	N + C	Esophagus, stomach, intestine, lymphocyte	✓	Caspase-1/4/5/11 cleavage	Innate immunity	Sepsis
DFNA5 (*Dfna5*)	7p15 (6B2.3)	N + C	Placenta, cochlea, brain, small intestine	✓	?	?	Deafness
DFNB59 (*Dfnb59/ Pejvakin*)	2q31.2 (2C3)	N	Inner ear, auditory pathway	?	N/A	?	Deafness

[a]N and C refer to the gasdermin-N and gasdermin-C domains, respectively.
[b]The expression data are taken from [30,31,54,55,58] as well as from BioGPS (www.biogps.org), which only indicates relatively high abundance in the tissues listed.

GSDMC, GSDMD, DFNA5, and DFNB59 (Table 1). Mice have no GSDMB but possess three GSDMAs (GSDMA1–3) and four GSDMCs (GSDMC1–4), which are encoded in the same locus on mouse chromosomes 11 and 15, respectively. Except for DFNB59, all of the gasdermins adopt a GSDMD-like two-domain architecture; the two domains are capable of binding to each other, as shown for GSDMA/GSDMA3, GSDMB, GSDMC, and GSDMD [29]. The gasdermin-N domains of GSDMA/GSDMA3, GSDMB, GSDMC, and DFNA5, but not the full-length protein, all can induce mammalian cell pyroptosis and kill bacteria [29,35]. Biochemical analyses of gasdermin-N domains of GSDMA3 and GSDMA have revealed a similar lipid binding and membrane disruption activity as that of GSDMD. The gasdermin-N of GSDMA3 readily oligomerizes and forms pores on phosphoinositide or cardiolipin-containing membranes; artificial interdomain cleavage of GSDMA3 or GSDMA can drive cells to undergo pyroptosis [35]. Thus, given the similar pore-forming activity in different gasdermins, we redefine pyroptosis as gasdermin-mediated programmed necrosis.

Recently, the crystal structure of GSDMA3 was determined, on which a highly similar structure of GSDMD was obtained from molecular modeling [35]. The overall structure is separated into the presumed gasdermin-N and gasdermin-C domains with a long loop harboring the inflammatory caspase cleavage site (Figure 1). The gasdermin-N domain has a core structure of an extended twisted β-sheet, while the gasdermin-C domain adopts a compact α-helical globular fold. The two domains are held together in close proximity through two major interdomain contacting sites, which buries approximately 30% of the surface area of each domain. Sequence homology suggests that the members of the gasdermin family (except for DFNB59) share similar 3D structures. Most of the autoinhibitory contacts seen in GSDMA3 are conserved in the family;

mutating the contacting residues in the gasdermin-C domain of GSDMD, GSDMA/GSDMA3, GSDMC, or DFNA5 leads to a constitutively active pyroptosis inducer. Thus, the gasdermin family of pyroptosis executioners shares a similar autoinhibited structure.

Other Gasdermin Family Members

GSDMA, GSDMB, GSDMC, and DFNA5 are different from GSDMD in that they lack the inflammatory caspase cleavage site, indicating other mechanisms of activation [29]. Once activated, most gasdermins will disrupt the plasma membrane, while certain gasdermin may also perforate some inner membranes to execute a nonpyroptosis function. In addition to pyroptosis induction, gasdermin pores may also serve as a protein secretion channel, like GSDMD pores for IL-1β secretion [28,29].

The physiological function of other gasdermins is largely unknown [31]. Gasdermins are highly expressed in the gastrointestinal tract (epithelium) and the skin system [40,41]. These early observations do not exclude a function of gasdermins in other biological systems. Genetic mutations of gasdermins give some clues for future studies of gasdermin-mediated pyroptosis. Among the GSDMA subfamily, *GSDMA* is frequently silenced in gastric cancers with a tumor growth factor-β-regulated growth-inhibition activity [42]. Mutation of *Gsdma3* causes epidermal hyperplasia, hyperkeratosis, and hair loss in mice [43–45]. *Gsdma3*-deficient mice show normal hair development [46], and heterozygous *Gsdma3* mutation causes the same alopecia phenotype. These observations suggest that alopecia is caused by a gain-of-function mutation in *Gsdma3* [47]. Nine *Gsdma3* mutations have been reported; two of them cause premature stop and loss of the gasdermin-C domain, and the other seven are missense mutations with spontaneous pyroptosis-inducing activity [29]. The seven mutations are all located in the interdomain contacting regions in the gasdermin-C domain of GSDMA3 [35]. Consistent with the constitutive pyroptosis induced by the mutant proteins, strong inflammatory responses in the skin and gradual depletion of the skin bulge stem cells are observed in *Gsdma3*-mutant mice [48,49]. Transgenic mice expressing *Gsdma1* harboring a mutation found in *Gsdma3* show the same skin defect [47]. Thus, one possible function of the GSDMA subfamily is to mediate immune defense in the skin through inducing pyroptosis. Similar to GSDMA and GSDMD, gasdermin-N domains of GSDMB and GSDMC harbor the same pyroptosis-inducing activity that is inhibited by their gasdermin-C domain [29,35]. Polymorphisms of *GSDMB* in humans are strongly associated with early childhood asthma [50–52], a disease characterized by chronic inflammation. A potential role of GSDMB and GSDMC in cancer progression has also been reported [41,53].

Genetic mutations of DFNA5 and DFNB59 cause nonsyndromic hearing loss in humans [54,55] (Table 1). All of the deafness mutations in *DFNA5* result in skipping exon 8, producing a truncated protein with a growth-inhibition activity [56]. As DFNA5 shares the biochemical properties of GSDMD, the deafness mutant should have spontaneous pyroptosis-inducing activity due to the loss of the gasdermin-C domain [35]. Consistent with the gain of function of the deafness mutation, *DFNA5* knockout mice exhibit no hearing impairment [57]. *DFNA5* is a transcriptional target of p53, indicating a cellular function in stress response [58]. DFNA5 is also a candidate tumor suppressor; its expression is silenced in some cancer cells by epigenetic modification of the promoter sequence [59,60]. DFNB59 (also known as pejvakin) is a unique gasdermin that lacks the gasdermin-C domain. Its gasdermin-N domain is most similar to that of DFNA5 (approximately 55% similarity). Different from *DFNA5*, the deafness mutations in *DFNB59* are autosomal recessive.

DFNA5 deafness is progressive, starting in the high frequencies at 5 to 10 years of age. The disease is sensorineural with functionally defective outer hair cells in the cochlea [57]. By contrast, the deafness manifestation in *DFNB59*-mutated individuals is heterogeneous; both

progressive and nonprogressive hearing loss is observed, and both auditory neuropathy and outer hair cell defects have been proposed as the cause of the deafness [55,61–64]. Supporting that *DFNA5* and *DFNB59* mutations cause deafness through different mechanisms, knockout of *DFNA5* cannot reverse the hearing impairment in knockin mice expressing a DFNB59 R183W deafness mutant [55]. A recent study shows that mice with homogenous *DFNB59* knockout exhibit heterogeneous hearing defects that are correlative to the level of sound exposure [65]. *DFNB59* deficiency renders both hair cells and auditory pathway neurons hypervulnerable to sound. Interestingly, DFNB59 was found to be associated with the peroxisome and regulate peroxisome proliferation. In *DFNB59*-deficient hair cells and auditory neurons, sound fails to stimulate DFNB59 expression, and the resulting peroxisome dysfunction causes oxidative stress and cell damage. Both missense and nonsense mutations are found in *DFNB59* deafness. The missense mutants (T54I, R183W, or C343S) and a truncation mutant devoid of the last 22 residues all fail to promote peroxisome proliferation, suggesting that these residues are essential for the biochemical activity of DFNB59. It is of great interest to investigate whether DFNB59 has pore-forming activity and how it regulates peroxisome function. While it is reasonable to accept that DFNA5-mediated pyroptosis in the cochlear system causes hearing loss, it is an open question whether DFNB59 functions as a pyroptosis factor in hearing system homeostasis. The expression profile of DFNA5 and DFNB59 also indicates that the two gasdermins may have other physiological functions besides those in the hearing system.

Conclusions and Perspectives

Studies on macrophage response to bacterial challenges in the 1980s and early 1990s record a form of cell death that is now realized to be pyroptosis. This cell death was often misregarded as apoptosis because of the involvement of caspase-1. For a long time, pyroptosis was thought to be an auxiliary event to IL-1β secretion, a critical inflammatory response in monocytes. Characterization of various inflammasomes has established the paramount importance of caspase-1 in innate immune defenses. The discovery of caspase-11 and caspase-4/5 function has expanded the notion of pyroptosis mediators from caspase-1 to the inflammatory caspase group, which also reveals that pyroptosis is not limited to monocytic cells.

Identification of GSDMD as a pyroptotic substrate of inflammatory caspases has revolutionized the understanding of pyroptosis. The membrane pore-forming activity in the gasdermin-N domain of GSDMD is responsible for pyroptosis execution, which carries out the necrotic function of pyroptosis. The large gasdermin family suggests ubiquitous occurrence of pyroptosis in mammals, and also prompts a new definition of pyroptosis as gasdermin-mediated programmed necrosis. The critical role of GSDMD in innate immunity and septic shock is just a start for appreciating the physiological functions of pyroptosis. Association of gasdermin mutations with various genetic diseases suggests a broad function of pyroptosis that is likely beyond immune defense. Given the unknown function of other gasdermins and their mechanism of activation, we are only at the tip of the iceberg to understand and appreciate the biological and pathological functions of pyroptosis (see Outstanding Questions). Lastly, the pore-forming mechanism shared by the gasdermin family provides an attractive target for future drug development in translational studies, including drugs for treating sepsis.

Outstanding Questions

Are there unknown inflammasome complexes that converge onto caspase-1 activation and pyroptosis induction?

What are the structural and biochemical mechanisms underlying membrane pore formation by the gasdermin-N domain?

How does the gasdermin pore induce cell swelling and other morphological changes characteristic of pyroptosis?

What are the cellular and physiological functions of other gasdermin-family members such as GSDMA, GSDMB, GSDMC, and DFNA5?

How do other gasdermins get activated to execute pyroptotic cell death?

How do DFNA5 and DFNB59 mutations cause deafness and do they function as pore-forming proteins or pyroptosis factors in this process?

Are there pyroptosis-independent functions of the gasdermin family of pore-forming proteins?

Does gasdermin-mediated pyroptosis function in biological processes other than innate immune defense?

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