nature immunology

Review article

https://doi.org/10.1038/s41590-023-01526-w

Molecular mechanisms of gasdermin D pore-forming activity

Received: 22 February 2023

Accepted: 3 May 2023

Published online: 5 June 2023

Check for updates

Pascal Devant & Jonathan C. Kagan

The regulated disruption of the plasma membrane, which can promote cell death, cytokine secretion or both is central to organismal health. The protein gasdermin D (GSDMD) is a key player in this process. GSDMD forms membrane pores that can promote cytolysis and the release of interleukin-1 family cytokines into the extracellular space. Recent discoveries have revealed biochemical and cell biological mechanisms that control GSDMD pore-forming activity and its diverse downstream immunological effects. Here, we review these multifaceted regulatory activities, including mechanisms of GSDMD activation by proteolytic cleavage, dynamics of pore assembly, regulation of GSDMD activities by posttranslational modifications, membrane repair and the interplay of GSDMD and mitochondria. We also address recent insights into the evolution of the gasdermin family and their activities in species across the kingdoms of life. In doing so, we hope to condense recent progress and inform future studies in this rapidly moving field in immunology.

Programmed cell death (PCD) has important roles in immune cell development and homeostasis, infection, autoimmunity and cancer. There are different forms of PCD, which can be classified as immunologically silent (such as apoptosis) or immunostimulatory (such as pyroptosis and necroptosis). A key difference between these forms of PCD is whether the plasma membrane of the dying cell becomes permeabilized. In the case of pyroptosis, the plasma membrane is permeabilized by proteins called gasdermins (GSDMs). Humans encode six GSDM proteins (GSDMA, GSDMB, GSDMC, GSDMD, GSDME and pejvakin) with considerable sequence and structural homology. GSDMD is the best-characterized member of this family, and is a key mediator of inflammasome-dependent pyroptotic cell death[1–3]. GSDMD is activated by proteolytic cleavage, which releases its N-terminal domain (NT-GSDMD) to form membrane pores[4–7]. These pores can serve as channels to mediate interleukin-1 (IL-1) family cytokine secretion, as well as ion exchange[8,9]. In instances where the extent of pore formation exceeds the membrane reparative capacity of the cell, lytic cell death (that is, pyroptosis) ensues[1,2]. General aspects of GSDMD biology, including its role in immune defense to infection and its therapeutic potential have been excellently reviewed elsewhere[10–14]. This Review is focused on biochemical and cell biological mechanisms that control GSDM activities in species that span the kingdoms of life.

Activation of GSDMD by inflammatory caspases

In resting human and mouse cells, GSDMD exists as an inactive pro-protein in the cytoplasm. GSDMD consists of two domains: the C-terminal domain (CT-GSDMD) and the NT-GSDMD, connected by a flexible interdomain linker. Binding of CT-GSDMD to NT-GSDMD keeps the protein in an auto-inhibited state that cannot form membrane pores. Pore-forming activity is achieved upon proteolytic cleavage at site Asp275 or Asp276 within the linker domain of human or mouse GSDMD, respectively[7,15]. Proteolytic activation of GSDMD is carried out primarily by a class of proteases known as inflammatory caspases, including caspase-1, caspase-4 and caspase-5 in humans as well as caspase-11 in mice. Caspase-1 becomes activated upon recruitment into an inflammasome. Inflammasomes are one of a growing number of supramolecular organizing centers (SMOCs), which represent the signaling organelles of the innate immune system[16]. Like all SMOCs, inflammasomes are not present in resting cells, but they are assembled upon infection or metabolic dysregulation of various sorts[17]. Caspase-4, caspase-5 and caspase-11 also cleave and activate GSDMD pore-forming activities, but these enzymes are not typically activated upon recruitment into inflammasomes. Rather, these caspases are activated to cleave GSDMD upon recognition of bacterial lipopolysaccharides (LPS) in the cytosol. This process can lead to the downstream

Division of Gastroenterology, Boston Children's Hospital and Harvard Medical School, Boston, MA, USA. e-mail: jonathan.kagan@childrens.harvard.edu

activation of the NLRP3 inflammasome through GSDMD-mediated K^+ efflux (often referred to as the noncanonical pathway of inflammasome activation)[18–20].

Dissection of enzyme–substrate interactions has revealed mechanisms of GSDMD cleavage by inflammatory caspases. Upon activation, caspases undergo autoprocessing at several sites (for example, Asp270 and Asp289 in caspase-4, or Asp285 in caspase-11) to remove an inhibitory interdomain linker. Cleavage at these sites generates the small (p10) catalytic subunit and facilitates formation of the enzymatically active caspase dimer[21]. This dimer can then bind to full-length GSDMD through a protease exosite interaction. X-ray crystallography has shown that the processed L2 loops in the caspase dimer form an intermolecular beta-sheet, harboring a cluster of conserved hydrophobic residues (Trp267 and Val291 in caspase-4 or Trp263 and Val287 in caspase-11), which inserts into a hydrophobic pocket in CT-GSDMD formed by Leu306, Leu310, Val367 and Leu370 (refs. 21,22). Moreover, a structure of caspase-1 bound to full-length GSDMD suggests the presence of a second binding interface between the interdomain linker of GSDMD and the peptide-binding groove near the caspase active site[22,23]. These interactions position the interdomain linker of GSDMD for cleavage. Although no contacts are observed between NT-GSDMD and the caspase in this structure, it was suggested that a RFWK motif in the β1–β2 loop of NT-GSDMD can bind and inhibit inflammatory caspases after cleavage, acting in a negative feedback loop[24]. Consistent with the structural evidence, biochemical experiments suggest that the amino acid residues directly downstream of the scissile peptide bond (called 'prime site' residues) in GSDMD impact substrate recognition, while cleavage is independent of the residues directly upstream of Asp276/Asp275 (the 'tetrapeptide')[21,25]. This exosite-mediated mode of substrate recognition appears to be specific to GSDMD, as mutations in the exosite have little effect on the cleavage of another prominent caspase-1 substrate, pro-IL-1β[26]. Mutations that modulate pro-IL-1β cleavage have little effect on GSDMD processing, suggesting that GSDMD and pro-IL-1β are recognized by distinct regions of caspase-1 (ref. 26).

Inflammatory caspase-independent activation

In addition to inflammatory caspases, other proteases can cleave GSDMD and activate its pore-forming activities (Fig. 1). For example, cathepsin G and elastase can generate a pore-forming NT-GSDMD fragment in neutrophils[27,28]. GSDMD activated in this manner is thought to contribute to the formation of neutrophil extracellular traps[29,30], although GSDMD-independent neutrophil extracellular trap formation also occurs[31,32]. The apoptotic initiator caspase-8 can cleave GSDMD in macrophages infected with pathogenic strains of *Yersinia*[33,34]. This activation is dependent on inhibition of the signaling cascade downstream of the MAP kinase kinase kinase TAK1 by the bacterial virulence factor YopJ[35,36]. Chemical inhibitors of TAK1, together with Toll-like receptor (TLR) or tumor necrosis factor (TNF) receptor activation, stimulate caspase-8-dependent GSDMD cleavage and pyroptosis[33,34]. The ability of caspase-8 to cleave GSDMD is context dependent. While caspase-8 efficiently cleaves GSDMD when activated through TNF complex IIb (as is the case after inhibition of TAK1), it poorly cleaves GSDMD downstream of TNF complex IIa or death-inducing signaling complex[37]. These signaling complexes form downstream of TNF receptor engagement in a cell-type and context-dependent manner, highlighting the pleiotropic effects of this cytokine on cell fate. Caspase-8 can also be activated by inflammasomes containing the adaptor protein ASC, especially in situations where activity of caspase-1 is absent, but whether caspase-8 cleaves GSDMD in this context is unknown[38,39]. The apoptotic effector caspases-3 and caspase-7 can also cleave GSDMD. However, while cleavage by caspase-8 produces a pore-forming NT-GSDMD fragment, cleavage by caspase-3 or caspase-7 occurs at a distinct site in NT-GSDMD (Asp87 in humans, Asp88 in mice), which blunts its pore-forming activities[40].

Beyond GSDMD, a number of proteases cleave and activate GSDM family members. For example, GSDMB is proteolytically activated by granzyme A in cells targeted by cytotoxic T cells or natural killer cells[41]. Contrary to its inhibitory effect on GSDMD, caspase-3 can activate GSDME to promote secondary necrosis during apoptotic signaling or induce pyroptosis in virus-infected keratinocytes[42,43]. The activation of GSDMC pore formation was linked to the activity of caspase-8 (refs. 44,45). Proteases expressed by infectious agents can also activate or inhibit GSDM pore-forming activities. For example, SpeB, a protease produced by group A *Streptococcus* can cleave GSDMA to cause pyroptosis in infected keratinocytes[46,47]. Similarly, Zika virus protease NS2B3 can cleave GSDMD at Ser250 to produce a cytolytic NT-GSDMD fragment[48]. These findings raise the possibility that GSDMs could be considered protease-sensing guard proteins, which stimulate effector-triggered immunity[49]. Conversely, certain viral proteases, such as severe acute respiratory syndrome coronavirus disease 2 3C-like protease or enterovirus 71 3C protease, can cleave internal sites in NT-GSDMD to inactivate its pore-forming activities, similar to caspase-3 and caspase-7 (refs. 50,51).

Structure and dynamics of the GSDMD pore

After proteolytic activation, NT-GSDMD exposes several patches of basic amino acids that serve as binding sites for acidic phospholipids, such as phosphoinositide phosphates, phosphatidylserine, phosphatidic acid and cardiolipin[4,7,52]. This binding facilitates NT-GSDMD recruitment to cellular membranes containing these lipids, including the inner leaflet of the plasma membrane, where it oligomerizes and forms multimeric pores[4,6,7]. The biophysical mechanisms that convert NT-GSDMD monomers into multimeric pores has been the subject of much investigation. Studies using cryo-electron microscopy (cryo-EM) demonstrated that the fully assembled GSDMD pore is composed of 31–34 NT-GSDMD subunits arranged in a ring with inner and outer diameters of 215 Å and 310 Å, respectively[53]. Oligomerization of GSDMD is mediated by lateral interfaces between neighboring GSDMD subunits, involving the α1 helices, which align head-to-tail to form a helical belt structure, and intensive hydrogen bonds between the transmembrane β-hairpins. This mode of oligomerization and interacting interfaces is conserved in all human GSDMs for which pore structures are available (namely GSDMD, GSDMA3 and GSDMB)[53–56]. The study describing the cryo-EM structure of the GSDMD pore also reported a structure of a GSDMD pre-pore with the same stoichiometry[53]. Membrane association of the GSDMD pre-pore and the pore is promoted by the membrane-proximal β1–β2 loop. This loop consists of a hydrophobic tip, which can insert into the membrane, and a basic patch that binds head groups of acidic membrane lipids. In the pre-pore, NT-GSDMD assumes a globular conformation similar to its auto-inhibited state[15,53]. When comparing the pre-pore to pore structure, it becomes evident that to form a transmembrane pore, NT-GSDMD must undergo a conformational change, inserting two β-hairpins into the membrane and thereby creating a intermolecular β-barrel with a large hydrophilic surface lining the interior of the pore[53]. Molecular dynamics simulations of GSDMD or murine GSDMA3 pores support a model whereby insertion of the β-hairpins occurs in a concerted fashion[57,58]. These simulations demonstrated that binding of the pre-pore to the membrane deforms the lipid bilayer and bends it into the pre-pore, thereby facilitating simultaneous insertion of the β-hairpins[57]. Transition from pre-pore to pore conformation in this manner would result in a lipid plug present in the interior of the pore. When fully assembled transmembrane pores were simulated with such a lipid plug present in the lumen, water molecules were attracted by the hydrophilic lining of the β-barrel. This change in hydration state caused the lipid plug to deform into a vesicle or bicelle-like structure. As a result, the lipids diffused away from the interior of the pore and membrane permeabilization is accomplished[57,58] (Fig. 2).

https://doi.org/10.1038/s41590-023-01526-w

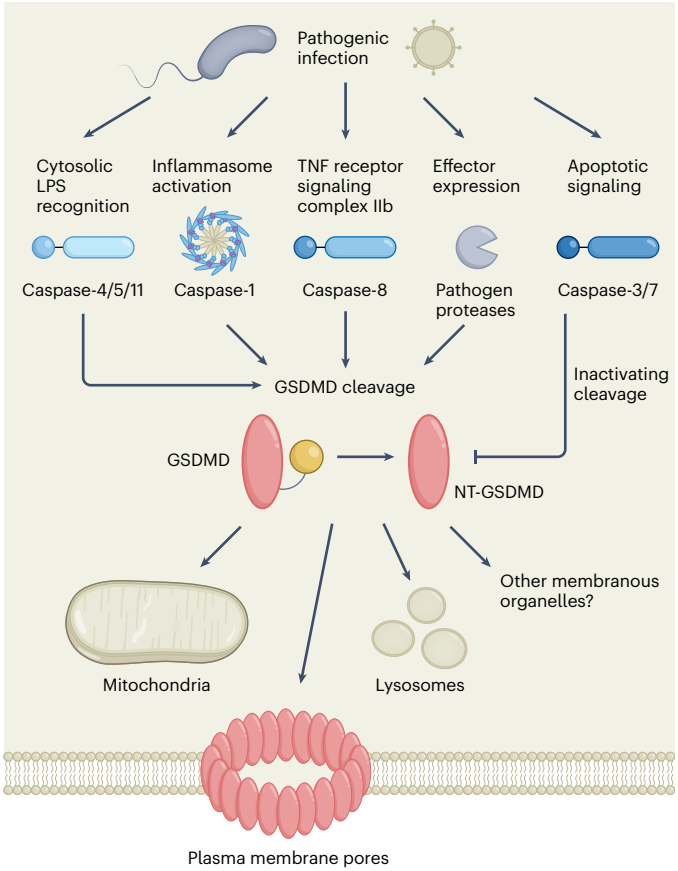

Fig. 1 | Cleavage of GSDMD by diverse cellular proteases activates its pore-forming activity. Pathogenic viral or bacterial infections trigger diverse innate immune pathways that activate proteases with the ability to activate GSDMD. Cytosolic LPS, a result of gram-negative bacterial infection, can be sensed by caspase-4, caspase-5 or caspase-11. Inflammasome activation can activate caspase-1. TNF receptor (TNFR) signaling (especially through TNF complex IIb) can activate caspase-8. Upon their activation, these proteases can cleave GSDMD within its interdomain linker connecting its C-terminal and N-terminal domains. In addition, proteases encoded by some infectious agents can similarly cleave and activate GSDMD. NT-GSDMD can then translocate to the plasma membrane, mitochondria, lysosomes and perhaps other membranous organelles and assemble into transmembrane pores. Apoptotic signaling can activate caspase-3 or caspase-7, which cleave GSDMD within its N terminus to inhibit its pore-forming abilities.

Atomic force microscopy imaging revealed that NT-GSDMD and NT-GSDMA3 can form not only ring-shaped, but also smaller arc-shaped and slit-shaped assemblies in supported lipid bilayers and liposomes[58,59]. All of these shapes were capable of forming transmembrane pores[57,59], with assemblies consisting of as few as two NT-GSDMD molecules able to form water-filled, ion-permeable channels in molecular dynamics simulations[57]. Time-resolved atomic frce microscopy and molecular dynamics simulations found that arc-shaped and slit-shaped pores can grow and fuse with each other[57–59]. Additionally, arc-shaped GSDMD oligomers can bend and exclude lipids from their interior, but might crack under the line tension of the open membrane edge to collapse into smaller slit-shaped transmembrane pores[57]. Once a pore has been assembled, electrophysiology and live-cell imaging has suggested that GSDMD pores may close and reopen multiple times over a period of minutes. Opening and closing kinetics were controlled by the phosphoinositide composition of the membrane[60]. The lipid microenvironment may therefore affect not only regulation pore assembly, but also pore function[57,60,61]. In conclusion, GSDMD pores are dynamic, heterogeneous structures and pore formation can likely proceed along variable pathways in parallel, such as concerted insertion of a pre-assembled ring-shaped pore into the membrane and plastic growth from smaller membrane-inserted oligomers (Fig. 2). The contribution of either assembly process to pore formation in the context of a cell is unknown. Data supporting either model were collected under specific conditions in vitro and in silico, and if either mechanism, or both, occur in vivo remains to be determined.

GSDMD pores as mediators of cytokine release

The formation of GSDMD pores can lead to the release of cytokines and other mediators of inflammation from the cell into the extracellular space. This release can occur in two major ways—through the transmembrane channel formed by GSDMD (that is, the pore) or after plasma membrane rupture. Size is one factor that determines whether a molecule can be released through GSDMD pores. Water molecules, ions and small chemicals (for example, fluorescent dyes), as well as small proteins such as IL-1β, IL-18, Rho GTPases, galectin-1 and nanobodies can diffuse from one side of a GSDMD-permeabilized membrane to another[8,9,62–65]. In contrast, larger molecules, such as the enzyme lactate dehydrogenase, the alarmin HMGB1, or high-molecular-weight dextrans, cannot[8,9,66,67]. The contribution of the different pore shapes and sizes described above to the release kinetics of different cargo remain to be determined, but it was estimated that an oligomer of ten or more NT-GSDMD subunits should be sufficient to permit passage of IL-1β[57].

Size is not the only variable that affects cargo release. Although the hydrodynamic radii of cleaved IL-1β is comparable to that of pro-IL-1β, the cleaved protein passes preferentially through GSDMD pores[53]. This finding can be explained by the presence of four acidic patches that create a negative potential in the interior of the GSDMD channel. This electrostatic filter permits the selective release of positively charged cargo (for example, IL-1β), while cargo with net negative charges (for example, pro-IL-1β) are repelled[53]. Mathematical modeling further predicts that interactions between GSDMD and acidic membrane lipids enhance charge selectivity of the pore, while salt diminishes electrostatic filtering by shielding electrostatic interactions[61]. Thus, intrinsic biophysical properties (size and charge), as well as extrinsic factors (lipid microenvironment, salt concentration) determine whether IL-1β can pass through a GSDMD pore. Other determinants of passage through GSDMD pores may exist, and studies of additional GSDMD cargoes will be informative.

While early studies focused on GSDMD-mediated secretion of IL-1β from phagocytes, recent work revealed roles for GSDMD and other GSDMs in the release of different IL-1 family cytokines from various cell types. For instance, release of IL-1α from macrophages after inflammasome activation depends on GSDMD, much like IL-1β[8]. However, unlike IL-1β, IL-1α is not directly cleaved by caspase-1. Instead, GSDMD pores allow for influx of extracellular calcium, which activates calpains that cleave IL-1α. IL-1α is then released through GSDMD pores[68]. IL-1α is also released from a subset of human T_H17 cells using a similar calcium-dependent process[69]. Following T cell receptor engagement, an NLRP3–caspase-8–caspase-3 axis activates GSDME, which forms plasma membrane pores, resulting in calcium influx, calpain activation and IL-1α processing and release. GSDMs therefore play a dual role in IL-1α maturation and secretion, acting upstream and downstream of IL-1α cleavage[68,69].

GSDMs have also been implicated in the release of the IL-1 family cytokine IL-33 (refs. 70,71). Exposure of macrophages and airway epithelial cell lines to allergen proteases leads to the cleavage of GSDMD in a caspase-independent manner to produce a noncanonical p40 fragment. This p40 fragment promotes the release of IL-33. Processing of GSDMD was dependent on allergen protease activity, although whether allergen proteases cleave GSDMD remains undefined[71]. GSDMD deficiency reduced IL-33 secretion and type 2 immunity-related

https://doi.org/10.1038/s41590-023-01526-w

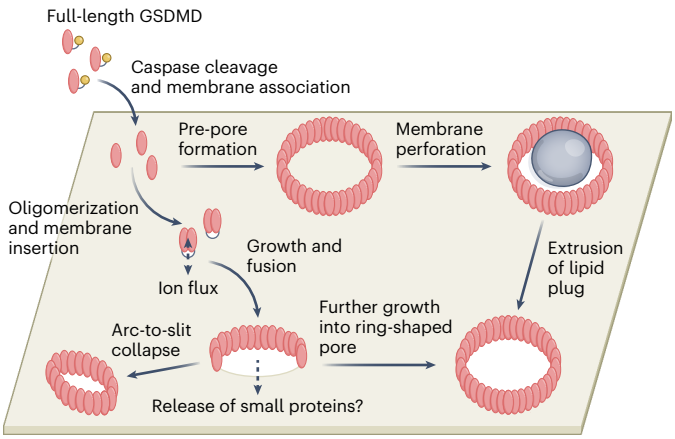

Fig. 2 | Assembly of the GSDMD pore can proceed along various paths. After proteolytic cleavage, NT-GSDMD monomers translocate and bind to the target membrane. NT-GSDMD monomers can then oligomerize into a membrane-associated ring-shaped pre-pore. Upon concerted insertion of the NT-GSDMD subunits into the membrane, the internal lipid plug will deform into a vesicle or bicelle, which leaves the now fully assembled transmembrane pore. Alternatively, individual NT-GSDMD subunits can insert into the membrane and assemble into smaller oligomers. Oligomers of two or more copies of NT-GSDMD will perforate the membrane and allow for ion fluxes. Smaller oligomers can assemble into arc-shaped pores, which may allow small proteins to traverse the membrane. Arc-shaped pores can collapse into smaller slit-shaped pores as a result of the line tension of the excluded lipid bilayer, or further grow and fuse into a full ring-shaped pore.

pathogenesis in mouse models of allergic airway inflammation[71]. In addition to its role in allergies, IL-33 impacts the development of hepatocellular carcinoma. In a mouse model of obesity-induced hepatocellular carcinoma, senescent hepatic stellate cells released IL-33 in a GSDMD-dependent manner[70]. In this context, GSDMD is processed by caspase-11, whereas IL-33 is cleaved into its bioactive fragment in the extracellular space by secreted elastases. IL-33 then activates regulatory T cells in the tumor microenvironment, which promotes tumorigenesis[70]. Inhibiting GSDMD pore formation with the drug disulfiram suppressed IL-33 release from hepatic stellate cells and hepatocellular carcinoma development, demonstrating the therapeutic potential of GSDMD-targeting drugs. Lastly, GSDMC-mediated IL-33 release has been described during helminth infection in the gut[72]. O-linked *N*-acetylglucosamine modification of the transcription factor STAT6 was reported to induce the upregulation of *Gsdmc* gene expression and GSDMC-mediated IL-33 release from intestinal epithelial cells. These processes contributed to the generation of a type 2 cytokine milieu and anti-helminth immunity[72].

GSDMD activities in living or dead cells

The formation of GSDMD pores was initially linked to the induction of pyroptosis, a lytic form of PCD[1,2]. Indeed, most inflammasome stimuli that are commonly used in the laboratory induce pyroptosis by a process dependent on GSDMD. However, the obligate link between GSDMD pore formation and pyroptosis has been challenged. For example, stimulation of dendritic cells and macrophages with TLR ligands and select oxidized phospholipids lead to the assembly of NLRP3 inflammasomes within living cells that retain the ability to execute phagocytosis[8,73,74]. Genetic analyses of phagocytes stimulated with TLR ligands and oxidized phospholipids indicated that the process of IL-1β release from living cells is dependent on NLRP3, ASC, inflammatory caspases and GSDMD[8,73,75]. In mice, these cells can also migrate to the lymph nodes while producing IL-1β[75]. Infection with a mutant strain of *Staphylococcus aureus* can also trigger IL-1 family cytokine release in the absence of cell death[8,76], and human primary dendritic cells treated with the NLRP3 agonist L18-MDP release IL-1β while remaining viable[77]. Similarly, neutrophils can survive and secrete IL-1β after infection with *Salmonella* or stimulation with NLRP3 agonists[78,79], and TLR4 signaling promotes IL-1β release from viable human, porcine and canine monocytes[26,80]. Other studies showed that both IL-1α and IL-1β can be released from living cells through GSDME pores[69,81]. Cell-death-independent IL-33 release through GSDMD pores has also been observed[70,71].

Before the above-described work, stimulated phagocytes were considered to be capable of living while they secrete cytokines other than IL-1β (such as TNF and IL-6), or releasing IL-1β upon pyroptosis. The finding that numerous stimuli and cell types can release IL-1β while maintaining viability suggests a third activation state in immune cells exists. In deference to the traditionally labeled state of macrophage and dendritic cell activation, where cytokines other than IL-1β are released upon stimulation, cells that have added IL-1β to the repertoire of secreted factors are considered to be hyperactivated[73,75]. Indeed, stimuli that hyperactivate dendritic cells lead to a more robust antigen-specific T cell response (and protective immunity to cancer) than stimuli that activate dendritic cells or induce pyroptosis[75]. These collective findings demonstrate that GSDM pores can serve as physiologically important conduits for IL-1 family cytokine release in various experimental settings.

In addition to the uncoupling of GSDMD pore formation from cell death through the study of cell hyperactivation mechanisms, GSDMD-mediated cell death has also been uncoupled from cell lysis. In wild-type cells, pyroptosis-inducing stimuli promote the formation of GSDMD pores that result in cell swelling and plasma membrane rupture (PMR)[63,64]. However, in murine and human macrophages lacking the plasma membrane-localized protein ninjurin-1 (NINJ1), GSDMD pore formation, IL-1β release, cell swelling and energetic cell death proceed normally, but PMR is defective[66,82]. NINJ1 had been characterized as a homophilic cell-adhesion molecule expressed in cells of the central nervous system, epithelial cells and immune cells[83–85]. However, a role in PMR and cell death had not been appreciated. The mechanism by which NINJ1 promotes PMR after cell death is undefined, but an amphipathic helix located at the N-terminus of NINJ1 is required for this activity. Moreover, PMR downstream of GSDMD pore formation correlates with the formation of NINJ1 oligomers[66]. An intriguing aspect of NINJ1 biology is that NINJ1-deficient mice display increased susceptibility to bacterial infections[66,86]. Additionally, NINJ1 is required for the egress of norovirus particles from infected cells[87]. These findings suggest that the process of cell lysis is important for host defense, either through the release of damage-associated molecular patterns or through an active role played by the cellular corpse. Pyroptotic corpses serve to trap intracellular bacteria and facilitate their elimination via recruited neutrophils[88]. It remains to be determined if NINJ1 contributes to the engulfment of pyroptotic corpses by neutrophils. The phenotypes associated with NINJ1 deficiency are analogous to cells treated with pyroptosis-inducing agents in the presence of the amino acid glycine[8,9,64,89]. Glycine has long been appreciated for its cytoprotective properties but its mechanism of action has been elusive[90]. These observations were linked biochemically by a report that found that glycine treatment inhibits the formation of NINJ1 oligomers that are typically associated with PMR[82]. These findings suggest that NINJ1 might be a target of glycine-mediated cytoprotection[82].

Removal of GSDMD pores from the cell membrane

Cells from diverse organisms have mechanisms that ensure membrane integrity. Cellular movement, environmental chemicals, bacterial pore-forming toxins, and T cell-derived and natural killer cell-derived perforin can all cause injuries to the plasma membrane. These injuries result in the influx of calcium from the extracellular space, which is detected by lysosomal calcium sensory proteins of the synaptotagmin family[91,92]. These proteins promote the fusion of

endosomes and lysosomes with the plasma membrane, resulting in the extrusion or endocytosis of the damaged site and a return to homeostasis[93]. The endolysosomal ESCRT-III machinery is a central regulator of synaptotagmin-mediated membrane repair, which acts to remove pores formed by GSDMD and the necroptosis-associated pore-forming protein MLKL[94,95]. Local delivery of ESCRT-III is mediated directly by GSDMD, as the pore formed is the source of the calcium influx that triggers recruitment of ESCRT-III to the cell surface. The ESCRT-III complex is recruited to the membrane, primed by the concave membrane around the GSDMD pore[53], and induces the formation of GSDMD-containing vesicles that bud off the cell surface[95]. Blocking ESCRT-III-mediated membrane repair, either by inhibiting calcium flux or by overexpressing a dominant-negative ESCRT-III component, amplified pyroptotic cell death and IL-1β release[95]. This connection between ESCRT-III and GSDMD may have therapeutic implications. In preclinical cancer models, the efficacy of bacteria-based delivery systems that induce GSDMD-mediated tumor cell pyroptosis was improved by co-delivering a calcium chelator to inhibit ESCRT-III-mediated membrane repair[96].

In addition to ESCRT-III-mediated repair, other mechanisms of GSDMD pore removal exist. After inflammasome activation, caspase-1 can activate caspase-7, which in turn cleaves and activates the lysosomal enzyme acid sphingomyelinase (ASM)[97]. Activation of ASM leads to the accumulation of ceramide, which induces enhanced endocytic membrane repair, thereby antagonizing GSDMD-mediated pyroptosis[97,98]. This type of membrane repair is critical to slow down pyroptotic cell death in intestinal epithelial cells upon *Salmonella* infection, thereby enabling the infected cell to be extruded from the epithelial barrier[97]. Whether this process is also activated in hyperactive cells to maintain viability is unclear.

Targeting of diverse organelles by GSDMD

The plasma membrane is considered the primary target membrane for GSDMD. However, GSDMD can also associate with other membranous organelles, including lysosomes and mitochondria (Fig. 2). Lysosomes have been implicated in the regulation of the NLRP3 inflammasome[99–101] and may serve as platforms for GSDMD processing during caspase-8-dependent pyroptosis[102]. In addition, lysosomal decay is a common feature of pyroptotic cell death, but whether this decay is explained by perforation of lysosomes by GSDMD has not been fully resolved[63,103]. Cleavage of the lysosomal enzyme ASM by cytosolic caspase-7 is suppressed in GSDMD-deficient cells, providing indirect evidence that GSDMD pores may permeabilize the lysosomal membrane in pyroptotic intestinal epithelial cells[97]. Direct targeting of lysosomes by GSDMD has been observed in neutrophils following inflammasome activation[104]. In this context, NT-GSDMD localizes to azurophilic granules and autophagosomes, resulting in lysosomal leakage, secondary cleavage of GSDMD by cathepsins and IL-1β release through an autophagy-related pathway[104].

The hypothesis that GSDMD might target mitochondria was formed due to the presence of the acidic lipid cardiolipin in the mitochondrial membrane. NT-GSDMD binds to cardiolipin[4,52] and targets mitochondria when activated by inflammatory caspases[105–108]. Other GSDM family members, such GSDME and GSDMA, are also able to perforate mitochondria[105,107,109]. Targeting of mitochondria by GSDMs results in their depolarization and contributes to the induction of reactive oxygen species (ROS) and energetic cell death during pyroptosis, which usually precedes PMR[63,64,106]. Moreover, mitochondrial GSDM pores can serve as conduits for cytochrome *c* to enter the cytosol, where it activates the apoptosome in a feed-forward loop[105].

If and to what degree GSDMs target mitochondria appears to depend on the specific protein, context and cell type. For example, GSDMA has a preference for associating with mitochondrial membranes over the plasma membrane, although the reason for this behavior is unclear[109]. GSDMD was reported to target mitochondria in murine endothelial cells following LPS transfection and in mouse models of septic shock[108]. Following inflammasome activation, macrophages derived from mice carrying a gain-of-function mutation in the gene encoding LRRK2, which has been associated with the development of diseases such as Parkinson's disease and Crohn's disease as well as increased susceptibility to mycobacterial infections (*Lrrk2*^{G2019S})[110–112], exhibited increased GSDMD-dependent cell death but decreased secretion of mature IL-1β[112]. This observation implies that pyroptosis may have been converted into a different type of PCD, thereby uncoupling cell death from caspase-1-dependent cytokine processing. Mechanistic analysis suggested that the aberrant production of mitochondrial ROS in *Lrrk2*^{G2019S} cells promoted recruitment of NT-GSDMD to mitochondria, which led to a burst in cellular ROS and activation of RIPK3-dependent, RIPK1-dependent and MLKL-dependent necroptosis[112]. Additional work is needed to understand how GSDMs target and form pores in diverse membranes, and whether repair mechanisms exist for all organelles disrupted by GSDM pores.

Posttranslational modifications of GSDMD

A number of studies suggest a connection between metabolism, ROS and GSDMD activities. While ROS can act at the apex of the inflammasome pathway by controlling priming and activation of the NLRP3 inflammasome[113,114], recent studies suggest a direct link between cellular redox state and GSDMD. As such, ROS was shown to promote cleavage of GSDMD by inflammatory caspases[115,116]. ROS production by xanthine oxidase and downstream signaling by the MAP kinases JNK2 and MAP3K5 was reported to promote trafficking of GSDMD to the plasma membrane[117]. ROS can also directly affect GSDMD after cleavage, at the level of oligomerization and pore formation. A genome-wide CRISPR–Cas9 loss-of-function screen identified components of the Ragulator–Rag–mTORC1 axis, a key regulatory pathway in cellular metabolism, as positive regulators of GSDMD pore formation[118]. Ragulator–Rag controls cellular ROS production and GSDMD oligomerization and pyroptosis[118]. Defects in GSDMD oligomerization and pyroptosis were complemented by the induction of cellular ROS through mitochondrial poisons or priming with microbial products[118,119]. Mechanistically, GSDMD senses ROS through the Cys192 residue in its N-terminal domain. Oxidation of this residue promotes oligomerization of GSDMD and pore formation[119]. This finding suggests that, like phosphorylation cascades in other signal transduction pathways, ROS acts at multiple stages of the inflammasome pathway (upstream and downstream of GSDMD cleavage) to regulate pore formation and pyroptosis.

Although the biochemical mechanism of how Cys192 oxidation promotes GSDMD oligomerization and pyroptosis is undefined, these studies highlight the importance of posttranslational modifications (PTMs) for GSDMD regulation (Table 1). We speculate that oxidation of Cys192 might aid in the removal or addition of another inhibitory or activating PTM, respectively. Palmitoylation of GSDMD at Cys192 was suggested to occur in a ROS-dependent manner[120,121]. Similarly, bacterial GSDM homologs are lipidated to assist membrane association and human GSDME can be palmitoylated in its C-terminal domain, which promotes pyroptosis by dissociating NT-GSDME from CT-GSDME after cleavage[122,123]. In addition, cysteine residues in GSDMD are the target of PTMs other than ROS to modulate its pore-forming activities. Itaconate, a Krebs cycle-derived metabolite that accumulates in myeloid cells upon prolonged TLR-stimulation, can modify Cys77 in murine GSDMD[124]. The specific effects of this PTM on GSDMD has not been explicitly determined, but together with the inhibitory itaconation of caspase-1 and NLRP3, it can tolerize cells to NLRP3 inflammasome activators[124,125]. Irreversible succination of Cys192 in GSDMD by fumarate also prevents GSDMD-mediated pyroptosis by disrupting the interaction between GSDMD and caspase-1 (ref. 126). Resulting anti-inflammatory effects can be mimicked by treating cells or mice with the drug dimethyl fumarate (DMF). Other small-molecule drugs such as disulfiram and necrosulfonamide also inhibit GSDMD by covalently binding to Cys192 (refs. 127,128). Disulfiram and DMF inhibit pore

Table 1 | PTMs affecting activities of GSDM family members

Protein	PTM	Modified residue	Effect	Ref.
GSDMD	Oxidation	Cys192 (only tested in mouse)	Promotes oligomerization and pore formation	119
GSDMD	Oxidation	Cys38, Cys56, Cys268, Cys467 (only tested in human)	Promotes cleavage by caspase-1	116
GSDMD	Disulfiram	Cys191/Cys192 (human/mouse)	Inhibits pore formation	127
GSDMD	Necrosulfamide	Cys191/Cys192 (human/mouse)	Inhibits pore formation	128
GSDMD	Succination (fumarate/DMF)	Cys191/Cys192 (human/mouse)	Inhibits cleavage by caspase-1 and pore formation	126
GSDMD	Itaconation	Cys77 (mouse-specific; not present in human)	Most likely inhibitory	124
GSDMD	Phosphorylation	Thr213 (only tested in human)	Inhibits pore formation	130
GSDMD	Lys27-linked polyubiquitination	Lys203, Lys204/Lys204, Lys205 (human/mouse)	Promotes pyroptosis	132
GSDMD	Lys63-linked/ Lys48-linked polyubiquitination	LysK55, Lys62, Lys203 (human-specific)	Targeting for degradation	55,56,133,135
GSDMD	Palmitoylation	Cys192	Promotes membrane association	120,121
GSDMA	Phosphorylation	Thr8 (only tested in human)	Inhibits pore formation	105
GSDMB	Lys48-linked polyubiquitination	At least Lys177, Lys190, Lys192 (only tested in human)	Targeting for degradation and direct inhibition of pore formation	55,56,133–135
GSDME	Phosphorylation	Thr6 (only tested in human)	Inhibits pore formation	105
GSDME	Disulfiram	Undefined	Inhibits pore formation	129
GSDME	Succination (fumarate/DMF)	Cys45	Inhibits pyroptosis	126
GSDME	Palmitoylation	Cys407/Cys408 (human/mouse)	Promotes pyroptosis	123
Bacterial GSDMs	Palmitoylation	Cys3/Cys3/Cys4/Cys7 (Runella/ Bradyrhizobium/ Vitiosangium/Lysobacter)	Promotes membrane permeabilization	122

formation by both GSDMD and GSDME[126,129]. This finding is notable as Cys192, their target in GSDMD, is not conserved in GSDME. While it was shown that succination targets Cys45 in GSDME[126], the target of disulfiram in GSDME is undefined.

Another possible role of cysteine PTMs in GSDMD is through disulfide bond formation. Indeed, a common way to study GSDMD pore formation is to analyze it by SDS–PAGE under denaturing but non-reducing conditions[4,118,119,128]. In this assay, GSDMD pores are detected as large, detergent-stable oligomers that are collapsed into a single monomer band when a reducing agent is added. This behavior is commonly observed for protein complexes stabilized by disulfide bonds and depends on the presence of cysteines in NT-GSDMD (our unpublished observation). However, the cryo-EM structure of the GSDMD pore is inconsistent with the formation of disulfide bonds[53]. Thus, there might be as-yet-undefined, reversible cysteine-linked PTMs that stabilize the GSDMD pore.

Cysteines are not the only residues in GSDMD that can be modified by PTMs. Phosphoproteomic analyses have determined that multiple threonine and serine residues in several human GSDMs can be phosphorylated[130,131]. The phosphorylation at Thr213 in GSDMD, Thr6 in GSDME or Thr8 in GSDMA prevent pore formation and pyroptosis, perhaps by interfering with oligomerization through steric hindrance[105,130]. The kinases responsible for the phosphorylation and the physiological relevance of these modifications are not well understood.

Finally, several sites in GSDMD have been found to be ubiquitinated. Addition of Lys27-linked polyubiquitination to Lys203 or Lys204 in human GSDMD (Lys204/Lys205 in mouse) by the E3 ubiquitin ligase synoviolin promotes GSDMD-mediated pyroptosis through an undefined mechanism[132]. In contrast, the *Shigella* effector IpaH7.8 decorates GSDMD and GSDMB with Lys48 and Lys63 polyubiquitin chains, targeting them for proteasomal degradation as a mechanism of immune evasion[133–135]. Pore formation by GSDMB, but not GSDMD, is directly inhibited by IpaH7.8-mediated ubiquitination in vitro, indicating additional proteasome-independent mechanisms of inhibition[55]. Structural studies revealed that the leucine-rich repeat domain of IpaH7.8 interacts with GSDMB through a cluster of negatively charged amino acids in its N-terminal domain[55,56,135]. Importantly, this interaction motif is conserved in human, but not murine GSDMD. As a result, IpaH7.8 can only target human GSDMD for degradation[55,56]. These species-specific differences in GSDMD may contribute to the higher susceptibility of humans to *Shigella* infection compared to mice[55,56,133,135]. Ubiquitin-mediated immune evasion of GSDMD has also been observed in cells infected with *Mycobacterium tuberculosis* (*Mtb*). Cellular ubiquitin acts as a cofactor for the *Mtb*-encoded phospholipase Ptpb, which dephosphorylates the membrane lipid PtdIns(4,5)P_2, thereby preventing plasma membrane localization of NT-GSDMD, IL-1β release and pyroptosis[136].

Activities of GSDMs across the tree of life

We are beginning to appreciate the remarkable conservation of GSDM-like pore-forming proteins, and other innate immune regulators, across the kingdoms of life[137]. It is believed that all GSDMs originated from a common ancestor. This proto-GSDM, which likely had the ability to form pores, evolved into the GSDM-like proteins found today in a variety of species, including vertebrates, invertebrates, fungi and even bacteria and archaea[122,138,139]. It is notable that GSDMs appear to be absent from plants, unlike other machineries involved in intracellular immune surveillance (such as NLR proteins) that are conserved in animals, plants and prokaryotes[140,141].

Phylogenetic and gene synteny analyses made it possible to reconstruct the evolutionary history of GSDMs in metazoans (Fig. 3a)[139,142]. Most invertebrates, such as Cnidaria and Mollusca, and early Chordata,

https://doi.org/10.1038/s41590-023-01526-w

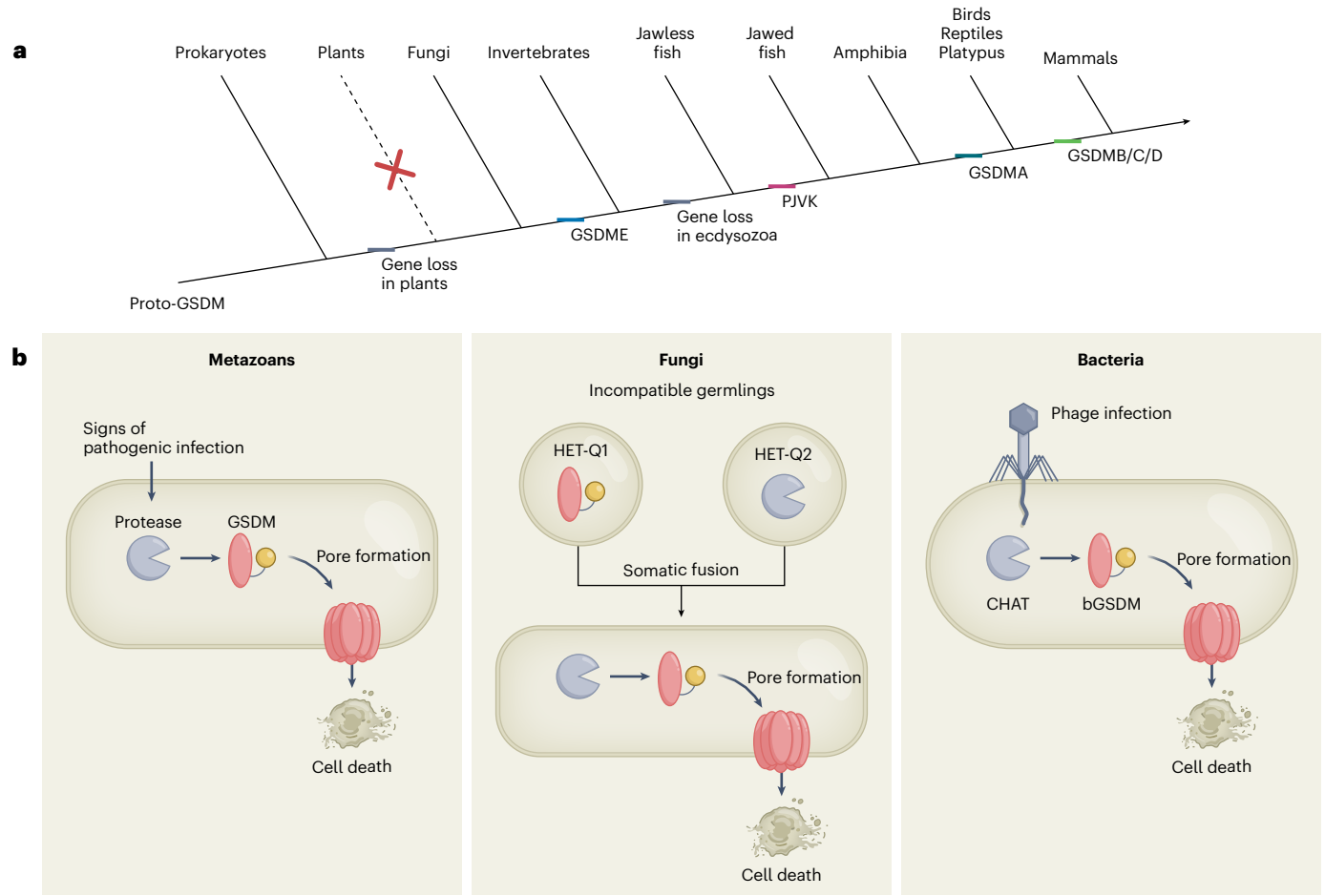

Fig. 3 | Evolutionary history of the GSDM protein family. a, All GSDMs are likely derived from one common ancestor. This pore-forming proto-GSDM diversified to give rise to all GSDM proteins known today in prokaryotes, fungi and metazoans. GSDME is the most ancient GSDM found in metazoans. Gene duplications in early vertebrates, amniotes and mammals resulted in the genes encoding pejvakin, GSDMA and GSDMB/GSDMC/GSDMD, respectively. GSDMs appear to be absent from plants and ecdysozoa (such as insects and nematodes). **b**, GSDMs from various kingdoms of life can be activated by proteolysis. GSDMs found in metazoans are typically cleaved by proteases such as caspases or granzymes upon pathogen infection. Fungal GSDMs (such as HET-Q1), which are involved in allorecognition, can be cleaved and activated by subtilisin-like proteases (such as HET-Q2). bGSDMs can be cleaved, for example, by caspase-like CHAT proteases, which are activated in response to phage infection. Proteolytic cleavage of these diverse GSDMs leads to membrane pore formation and cell death.

such as the jawless fish lamprey and lancelets, possess one *GSDM* gene, which is most similar to mammalian *GSDME*. Members of the ecdysozoa clade of invertebrates (including insects and nematodes) do not encode this *GSDM* gene[139]. In jawed fish, the ancestral *GSDM* gene underwent a gene duplication event, giving rise to the genes encoding GSDME and the non-pore-forming pejvakin. Some jawed fish have up to three *GSDME* homologs (*GSDMEa*, *GSDMEb*, *GSDMEc*)[143]. Birds, reptiles and platypus encode one additional *GSDM* gene, *GSDMA*, which originated from another duplication of *GSDME*. In mammals, *GSDMA* duplicated three more times, giving rise to genes for *GSDMB*, *GSDMC* and *GSDMD*, all of which display immune functions[139,142]. The diversification of *GSDM* genes can therefore be seen as a sign of the increased sophistication of the evolving mammalian immune system.

Some GSDM proteins from species with increasing evolutionary distance to humans have been characterized functionally. For example, *GSDME* homologs from ducks and teleost fish are cleaved by caspases to induce lytic cell death[143–146]. In the corals *Orbicella faveolata* and *Pocillopora damicornis* a *GSDM* gene most similar to human *GSDME* exists[147]. Coral GSDME is cleaved by coral and human caspase-3, forms membrane pores that contributes to necrosis and organismal death when these animals are infected with a bacterial pathogen[147]. These findings show that pyroptotic cell death is a defense mechanism in diverse clades of metazoans.

GSDM-like proteins from fungi have been implicated in the process of allorecognition. Allorecognition occurs after somatic fusion of germlings from two incompatible strains of fungus and leads to the elimination of resulting heterokaryons. GSDM-like proteins from two strains of fungus have been characterized, the RCD-1 proteins from *Neoforma crassa* (encoded by the genes *rcd-1-1* and *rcd-1-2*) and the HET-Q proteins (encoded by the idiomorphic genes *het-Q1* and *het-Q2*) from *Podospora anserina*[148–150]. When germlings from one fungal strain express one component of these allorecognition systems and germlings from another strain express the other, the resulting heterokaryons undergo lytic cell death[148,150]. RCD-1-1 and RCD-1-2 from *N. crassa* are homologous to the N-terminal pore-forming domain of mammalian GSDMs[149]. When coexpressed in mammalian cells, the two proteins form membrane-bound clusters, indicative of membrane pores, which cause pyroptosis-like cell death[149]. These findings indicate that coexpression of the two RCD-1 proteins is sufficient to induce lytic death, but the underlying biochemical mechanisms of this process are not understood. HET-Q1 from *P. anserina* shares homology to mammalian GSDMs and includes a short C-terminal domain. HET-Q2 is a

subtilisin-like serine protease that can cleave HET-Q1 to liberate its N-terminal pore-forming domain and trigger lytic cell death. Thus, HET-Q1 is proteolytically activated by HET-Q2, similar to how GSDMs in metazoans are activated by caspases[150] (Fig. 3b). Many fungal *GSDM* genes are located near HET-Q2-like protease genes in the genome, suggesting that proteolytic activation of fungal GSDMs may be widespread. Moreover, HET-Q2-like proteases sometimes contain immune-related domains akin to those found in NLRs[150]. These findings suggest that GSDM-mediated cell death pathways in fungi could have a role beyond allorecognition, for example, in immunity.

Despite limited sequence conservation between GSDMs from prokaryotes and mammals, they share structural homology in their pore-forming N-terminal domains[122]. Similar to fungal GSDMs, bacterial GSDMs (bGSDMs) lack a large C-terminal domain and instead contain a short hydrophobic C-terminal peptide. Within bacterial genomes, GSDM-like genes cluster with genes encoding caspase-like CHAT proteases, which can cleave off this C-terminal peptide to release auto-inhibition[122]. Cleaved bGSDMs form membrane pores of varying size in liposomes in vitro and permeabilize membranes when expressed in bacterial cells[122]. Operons containing bGSDM and CHAT protease genes are located within genomes next to antiphage defense systems. Heterologous expression of the GSDM-containing operons in *Escherichia coli* attenuated phage infection, suggesting a role for bGSDMs in antiphage immunity (Fig. 3b)[122]. Taken together, these studies demonstrate that GSDMs are widespread across the tree of life and despite large phylogenetic distances, they share many functional characteristics, such as activation by proteolysis, pore-forming activity and the fold of the pore-forming domain. They also have roles in immune-related processes, tailored to the specific needs of each organism.

Concluding remarks

While much progress has been made in understanding the molecular mechanisms that regulate GSDMD pore formation, many open questions remain, particularly how the various regulatory mechanisms are coordinated in time and space. For instance, structural studies have characterized the interactions between inflammatory caspases and GSDMD in vitro. Yet, it is unclear how these enzyme–substrate complexes are formed in the three-dimensional space of a cell and what additional factors are required to coordinate trafficking of GSDMD to desired subcellular localizations before and after cleavage. The interplay between GSDMD, cellular redox state and mitochondria provides another intriguing example. The study of Ragulator–Rag-deficient cells has established that ROS are required for the formation of GSDMD pores. At the same time, depolarization of mitochondria by GSDMD pores amplifies ROS levels. How these events are linked is unknown. Basal ROS may license the formation of a low level of GSDMD pores in the mitochondria early during pyroptosis, resulting in a burst of ROS and escalating formation of GSDMD pores in the plasma membrane, eventually killing the cell.

Additional mechanistic insight into GSDMD biology will inform discussions of therapeutic intervention. Several small-molecule inhibitors of GSDMD have been identified; however, these inhibitors suffer from poor specificity and adverse effects. In addition to targeting GSDMD directly, the identification of compounds modulating cell biological processes and PTMs that control GSDMD pores will be a promising approach for drug development. Specific inhibitors or activators of these processes might allow for the fine-tuning of GSDMD activities, for example, by specifically generating GSDMD pores of different sizes (rings versus slits versus arcs) to control secretion of certain cargo but not others. Further study of GSDMs from various species will inform these efforts as evolutionarily conserved properties of GSDMs may present particularly promising therapeutic targets.

References

1. Shi, J. et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* **526**, 660–665 (2015).
2. Kayagaki, N. et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* **526**, 666–671 (2015).
3. He, W. et al. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res.* **25**, 1285–1298 (2015).
4. Liu, X. et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* **535**, 153–158 (2016).
5. Aglietti, R. A. et al. GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc. Natl Acad. Sci. USA* **113**, 7858–7863 (2016).
6. Sborgi, L. et al. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J.* **35**, 1766–1778 (2016).
7. Ding, J. et al. Pore-forming activity and structural auto-inhibition of the gasdermin family. *Nature* **535**, 111–116 (2016).
8. Evavold, C. L. et al. The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. *Immunity* **48**, 35–44 (2018).
9. Heilig, R. et al. The gasdermin-D pore acts as a conduit for IL-1β secretion in mice. *Eur. J. Immunol.* **48**, 584–592 (2018).
10. Orning, P., Lien, E. & Fitzgerald, K. A. Gasdermins and their role in immunity and inflammation. *J. Exp. Med.* **216**, 2453–2465 (2019).
11. Vora, S. M., Lieberman, J. & Wu, H. Inflammasome activation at the crux of severe COVID-19. *Nat. Rev. Immunol.* **21**, 694–703 (2021).
12. Booty, L. M. & Bryant, C. E. Gasdermin D and beyond—gasdermin-mediated pyroptosis in bacterial infections. *J. Mol. Biol.* **434**, 167409 (2022).
13. Liu, X., Xia, S., Zhang, Z., Wu, H. & Lieberman, J. Channelling inflammation: gasdermins in physiology and disease. *Nat. Rev. Drug Discov.* **20**, 384–405 (2021).
14. Broz, P., Pelegrín, P. & Shao, F. The gasdermins, a protein family executing cell death and inflammation. *Nat. Rev. Immunol.* **20**, 143–157 (2020).
15. Kuang, S. et al. Structure insight of GSDMD reveals the basis of GSDMD auto-inhibition in cell pyroptosis. *Proc. Natl Acad. Sci. USA* **114**, 10642–10647 (2017).
16. Kagan, J. C., Magupalli, V. G. & Wu, H. SMOCs: supramolecular organizing centres that control innate immunity. *Nat. Rev. Immunol.* **14**, 821–826 (2014).
17. Schroder, K. & Tschopp, J. The inflammasomes. *Cell* **140**, 821–832 (2010).
18. Shi, J. et al. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* **514**, 187–192 (2014).
19. Baker, P. J. et al. NLRP3 inflammasome activation downstream of cytoplasmic LPS recognition by both caspase-4 and caspase-5. *Eur. J. Immunol.* **45**, 2918–2926 (2015).
20. Rühl, S. & Broz, P. Caspase-11 activates a canonical NLRP3 inflammasome by promoting K^+ efflux. *Eur. J. Immunol.* **45**, 2927–2936 (2015).
21. Wang, K. et al. Structural mechanism for GSDMD targeting by autoprocessed caspases in pyroptosis. *Cell* **180**, 941–955 (2020).
22. Liu, Z. et al. Caspase-1 engages full-length gasdermin D through two distinct interfaces that mediate caspase recruitment and substrate cleavage. *Immunity* **53**, 106–114 (2020).
23. Yang, J. et al. Mechanism of gasdermin D recognition by inflammatory caspases and their inhibition by a gasdermin D-derived peptide inhibitor. *Proc. Natl Acad. Sci. USA* **115**, 6792–6797 (2018).

24. Hu, Y. et al. The gasdermin D N-terminal fragment acts as a negative feedback system to inhibit inflammasome-mediated activation of caspase-1/11. *Proc. Natl Acad. Sci. USA* **119**, e2210809119 (2022).
25. Bibo-Verdugo, B., Snipas, S. J., Kolt, S., Poreba, M. & Salvesen, G. S. Extended subsite profiling of the pyroptosis effector protein gasdermin D reveals a region recognized by inflammatory caspase-11. *J. Biol. Chem.* **295**, 11292–11302 (2020).
26. Devant, P., Cao, A. & Kagan, J. C. Evolution-inspired redesign of the LPS receptor caspase-4 into an interleukin-1β–converting enzyme. *Sci. Immunol.* **6**, eabh3567 (2021).
27. Kambara, H. et al. Gasdermin D exerts anti-inflammatory effects by promoting neutrophil death. *Cell Rep.* **22**, 2924–2936 (2018).
28. Burgener, S. S. et al. Cathepsin G inhibition by Serpinb1 and Serpinb6 prevents programmed necrosis in neutrophils and monocytes and reduces GSDMD-driven inflammation. *Cell Rep.* **27**, 3646–3656 (2019).
29. Sollberger, G. et al. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. *Sci. Immunol.* **3**, eaar6689 (2018).
30. Chen, K. W. et al. Noncanonical inflammasome signaling elicits gasdermin D-dependent neutrophil extracellular traps. *Sci. Immunol.* **3**, eaar6676 (2018).
31. Stojkov, D. et al. NET formation is independent of gasdermin D and pyroptotic cell death. *Sci. Signal.* **16**, eabm0517 (2023).
32. Chauhan, D. et al. GSDMD drives canonical inflammasome-induced neutrophil pyroptosis and is dispensable for NETosis. *EMBO Rep.* **23**, e54277 (2022).
33. Sarhan, J. et al. Caspase-8 induces cleavage of gasdermin D to elicit pyroptosis during *Yersinia* infection. *Proc. Natl Acad. Sci. USA* **115**, E10888–E10897 (2018).
34. Orning, P. et al. Pathogen blockade of TAK1 triggers caspase-8-dependent cleavage of gasdermin D and cell death. *Science* **362**, 1064–1069 (2018).
35. Palmer, L. E., Pancetti, A. R., Greenberg, S. & Bliska, J. B. YopJ of *Yersinia* spp. is sufficient to cause downregulation of multiple mitogen-activated protein kinases in eukaryotic cells. *Infect. Immun.* **67**, 708–716 (1999).
36. Rosadini, C. V. et al. A single bacterial immune evasion strategy dismantles both MyD88 and TRIF signaling pathways downstream of TLR4. *Cell Host Microbe* **18**, 682–693 (2015).
37. Demarco, B. et al. Caspase-8–dependent gasdermin D cleavage promotes antimicrobial defense but confers susceptibility to TNF-induced lethality. *Sci. Adv.* **6**, eabc3465 (2020).
38. Mascarenhas, D. P. A. et al. Inhibition of caspase-1 or gasdermin-D enable caspase-8 activation in the Naip5/NLRC4/ASC inflammasome. *PLoS Pathog.* **13**, e1006502 (2017).
39. Schneider, K. S. et al. The inflammasome drives GSDMD-independent secondary pyroptosis and IL-1 release in the absence of caspase-1 protease activity. *Cell Rep.* **21**, 3846–3859 (2017).
40. Taabazuing, C. Y., Okondo, M. C. & Bachovchin, D. A. Pyroptosis and apoptosis pathways engage in bidirectional crosstalk in monocytes and macrophages. *Cell Chem. Biol.* **24**, 507–514 (2017).
41. Zhou, Z. et al. Granzyme A from cytotoxic lymphocytes cleaves GSDMB to trigger pyroptosis in target cells. *Science* **368**, eaaz7548 (2020).
42. Wang, Y. et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. *Nature* **547**, 99–103 (2017).
43. Orzalli, M. H. et al. Virus-mediated inactivation of anti-apoptotic Bcl-2 family members promotes Gasdermin-E-dependent pyroptosis in barrier epithelial cells. *Immunity* **54**, 1447–1462 (2021).
44. Zhang, J. et al. The metabolite α-KG induces GSDMC-dependent pyroptosis through death receptor 6-activated caspase-8. *Cell Res.* **31**, 980–997 (2021).
45. Hou, J. et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nat. Cell Biol.* **22**, 1264–1275 (2020).
46. Deng, W. et al. Streptococcal pyrogenic exotoxin B cleaves GSDMA and triggers pyroptosis. *Nature* **602**, 496–502 (2022).
47. LaRock, D. L. et al. Group A *Streptococcus* induces GSDMA-dependent pyroptosis in keratinocytes. *Nature* **605**, 527–531 (2022).
48. Kao, Y. -T. et al. Zika virus cleaves GSDMD to disseminate prognosticable and controllable oncolysis in a human glioblastoma cell model. *Mol. Ther. Oncolytics* **28**, 104–117 (2023).
49. Remick, B. C., Gaidt, M. M. & Vance, R. E. Effector-triggered immunity. *Annu. Rev. Immunol.* **41**, 453–481 (2023).
50. Lei, X. et al. Enterovirus 71 Inhibits pyroptosis through cleavage of gasdermin D. *J. Virol.* **91**, e01069–17 (2017).
51. Planès, R. et al. Human NLRP1 is a sensor of pathogenic coronavirus 3CL proteases in lung epithelial cells. *Mol. Cell* **82**, 2385–2400 (2022).
52. Liu, Z. et al. Crystal structures of the full-length murine and human gasdermin D reveal mechanisms of auto-inhibition, lipid binding, and oligomerization. *Immunity* **51**, 43–49 (2019).
53. Xia, S. et al. Gasdermin D pore structure reveals preferential release of mature interleukin-1. *Nature* **593**, 607–611 (2021).
54. Ruan, J., Xia, S., Liu, X., Lieberman, J. & Wu, H. Cryo-EM structure of the gasdermin A3 membrane pore. *Nature* **557**, 62–67 (2018).
55. Wang, C. et al. Structural basis for GSDMB pore formation and its targeting by IpaH7.8. *Nature* **616**, 590–597 (2023).
56. Zhong, X. et al. Structural mechanisms for regulation of GSDMB pore-forming activity. *Nature* **616**, 598–605 (2023).
57. Schaefer, S. L. & Hummer, G. Sublytic gasdermin-D pores captured in atomistic molecular simulations. *Elife* **11**, e81432 (2022).
58. Mari, S. A. et al. Gasdermin-A3 pore formation propagates along variable pathways. *Nat. Commun.* **13**, 2609 (2022).
59. Mulvihill, E. et al. Mechanism of membrane pore formation by human gasdermin-D. *EMBO J.* **37**, e98321 (2018).
60. Santa Cruz Garcia, A. B., Schnur, K. P., Malik, A. B. & Mo, G. C. H. Gasdermin D pores are dynamically regulated by local phosphoinositide circuitry. *Nat. Commun.* **13**, 52 (2022).
61. Xie, W. J., Xia, S., Warshel, A. & Wu, H. Electrostatic influence on IL-1 transport through the GSDMD pore. *Proc. Natl Acad. Sci. USA* **119**, e2120287119 (2022).
62. Russo, A. J. et al. Intracellular immune sensing promotes inflammation via gasdermin D–driven release of a lectin alarmin. *Nat. Immunol.* **22**, 154–165 (2021).
63. de Vasconcelos, N. M., Van Opdenbosch, N., Van Gorp, H., Parthoens, E. & Lamkanfi, M. Single-cell analysis of pyroptosis dynamics reveals conserved GSDMD-mediated subcellular events that precede plasma membrane rupture. *Cell Death Differ.* **26**, 146–161 (2019).
64. DiPeso, L., Ji, D. X., Vance, R. E. & Price, J. V. Cell death and cell lysis are separable events during pyroptosis. *Cell Death Discov.* **3**, 17070 (2017).
65. Bertheloot, D. et al. Nanobodies dismantle post-pyroptotic ASC specks and counteract inflammation in vivo. *EMBO Mol. Med.* **14**, e15415 (2022).
66. Kayagaki, N. et al. NINJ1 mediates plasma membrane rupture during lytic cell death. *Nature* **591**, 131–136 (2021).
67. Volchuk, A., Ye, A., Chi, L., Steinberg, B. E. & Goldenberg, N. M. Indirect regulation of HMGB1 release by gasdermin D. *Nat. Commun.* **11**, 4561 (2020).

68. Tsuchiya, K. et al. Gasdermin D mediates the maturation and release of IL-1α downstream of inflammasomes. *Cell Rep.* **34**, 108887 (2021).
69. Chao, Y.-Y. et al. Human T_H17 cells engage gasdermin E pores to release IL-1α on NLRP3 inflammasome activation. *Nat. Immunol.* **24**, 295–308 (2023).
70. Yamagishi, R. et al. Gasdermin D–mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. *Sci. Immunol.* **7**, eabl7209 (2022).
71. Chen, W. et al. Allergen protease-activated stress granule assembly and gasdermin D fragmentation control interleukin-33 secretion. *Nat. Immunol.* **23**, 1021–1030 (2022).
72. Zhao, M. et al. Epithelial STAT6 O-GlcNAcylation drives a concerted anti-helminth alarmin response dependent on tuft cell hyperplasia and gasdermin C. *Immunity* **55**, 623–638 (2022).
73. Zanoni, I. et al. An endogenous caspase-11 ligand elicits interleukin-1 release from living dendritic cells. *Science* **352**, 1232–1236 (2016).
74. Zanoni, I., Tan, Y., Di Gioia, M., Springstead, J. R. & Kagan, J. C. By capturing inflammatory lipids released from dying cells, the receptor CD14 induces inflammasome-dependent phagocyte hyperactivation. *Immunity* **47**, 697–709 (2017).
75. Zhivaki, D. et al. Inflammasomes within hyperactive murine dendritic cells stimulate long-lived T cell-mediated anti-tumor immunity. *Cell Rep.* **33**, 108381 (2020).
76. Wolf, A. J. et al. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell* **166**, 624–636 (2016).
77. Hatscher, L. et al. Select hyperactivating NLRP3 ligands enhance the T_H1- and T_H17-inducing potential of human type 2 conventional dendritic cells. *Sci. Signal.* **14**, eabe1757 (2021).
78. Chen, K. W. et al. The neutrophil NLRC4 inflammasome selectively promotes IL-1β maturation without pyroptosis during acute salmonella challenge. *Cell Rep.* **8**, 570–582 (2014).
79. Boucher, D. et al. Caspase-1 self-cleavage is an intrinsic mechanism to terminate inflammasome activity. *J. Exp. Med.* **215**, 827–840 (2018).
80. Gaidt, M. M. et al. Human monocytes engage an alternative inflammasome pathway. *Immunity* **44**, 833–846 (2016).
81. Zhou, B. & Abbott, D. W. Gasdermin E permits interleukin-1 beta release in distinct sublytic and pyroptotic phases. *Cell Rep.* **35**, 108998 (2021).
82. Borges, J. P. et al. Glycine inhibits NINJ1 membrane clustering to suppress plasma membrane rupture in cell death. *Elife* **11**, e78609 (2022).
83. Araki, T. & Milbrandt, J. Ninjurin, a novel adhesion molecule, is induced by nerve injury and promotes axonal growth. *Neuron* **17**, 353–361 (1996).
84. Araki, T., Zimonjic, D. B., Popescu, N. C. & Milbrandt, J. Mechanism of homophilic binding mediated by ninjurin, a novel widely expressed adhesion molecule. *J. Biol. Chem.* **272**, 21373–21380 (1997).
85. Odoardi, F. et al. T cells become licensed in the lung to enter the central nervous system. *Nature* **488**, 675–679 (2012).
86. Bjanes, E. et al. Genetic targeting of Card19 is linked to disrupted NINJ1 expression, impaired cell lysis, and increased susceptibility to *Yersinia* infection. *PLoS Pathog.* **17**, e1009967 (2021).
87. Wang, G., Zhang, D., Orchard, R. C., Hancks, D. C. & Reese, T. A. Norovirus MLKL-like protein initiates cell death to induce viral egress. *Nature* **616**, 152–158 (2023).
88. Jorgensen, I., Zhang, Y., Krantz, B. A. & Miao, E. A. Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis. *J. Exp. Med.* **213**, 2113–2128 (2016).
89. Fink, S. L. & Cookson, B. T. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell. Microbiol.* **8**, 1812–1825 (2006).
90. Weinberg, J. M., Bienholz, A. & Venkatachalam, M. A. The role of glycine in regulated cell death. *Cell. Mol. Life Sci.* **73**, 2285–2308 (2016).
91. Andrews, N. W. & Corrotte, M. Plasma membrane repair. *Curr. Biol.* **28**, R392–R397 (2018).
92. Cooper, S. T. & McNeil, P. L. Membrane repair: mechanisms and pathophysiology. *Physiol. Rev.* **95**, 1205–1240 (2015).
93. McCullough, J., Frost, A. & Sundquist, W. I. Structures, functions and dynamics of ESCRT-III/Vps4 membrane remodeling and fission complexes. *Annu. Rev. Cell Dev. Biol.* **34**, 85–109 (2018).
94. Gong, Y.-N. et al. ESCRT-III acts downstream of MLKL to regulate necroptotic cell death and its consequences. *Cell* **169**, 286–300 (2017).
95. Rühl, S. et al. ESCRT-dependent membrane repair negatively regulates pyroptosis downstream of GSDMD activation. *Science* **362**, 956–960 (2018).
96. Li, Z. et al. Enhancing gasdermin-induced tumor pyroptosis through preventing ESCRT-dependent cell membrane repair augments antitumor immune response. *Nat. Commun.* **13**, 6321 (2022).
97. Nozaki, K. et al. Caspase-7 activates ASM to repair gasdermin and perforin pores. *Nature* **606**, 960–967 (2022).
98. Tam, C. et al. Exocytosis of acid sphingomyelinase by wounded cells promotes endocytosis and plasma membrane repair. *J. Cell Biol.* **189**, 1027–1038 (2010).
99. Hornung, V. et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* **9**, 847–856 (2008).
100. Gaidt, M. M. et al. The DNA inflammasome in human myeloid cells is initiated by a STING-cell death program upstream of NLRP3. *Cell* **171**, 1110–1124 (2017).
101. Muñoz-Planillo, R. et al. K^+ efflux Is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity* **38**, 1142–1153 (2013).
102. Zheng, Z. et al. The lysosomal Rag–Ragulator complex licenses RIPK1– and caspase-8–mediated pyroptosis by *Yersinia*. *Science* **372**, eabg0269 (2021).
103. Averette, K. M. et al. Anthrax lethal toxin induced lysosomal membrane permeabilization and cytosolic cathepsin release Is Nlrp1b/Nalp1b-dependent. *PLoS ONE* **4**, e7913 (2009).
104. Karmakar, M. et al. N-GSDMD trafficking to neutrophil organelles facilitates IL-1β release independently of plasma membrane pores and pyroptosis. *Nat. Commun.* **11**, 2212 (2020).
105. Rogers, C. et al. Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. *Nat. Commun.* **10**, 1689 (2019).
106. Platnich, J. M. et al. Shiga toxin/lipopolysaccharide activates caspase-4 and Gasdermin D to trigger mitochondrial reactive oxygen species upstream of the NLRP3 inflammasome. *Cell Rep.* **25**, 1525–1536 (2018).
107. Torre-Minguela, C., Gómez, A. I., Couillin, I. & Pelegrín, P. Gasdermins mediate cellular release of mitochondrial DNA during pyroptosis and apoptosis. *FASEB J.* **35**, e21757 (2021).
108. Huang, L. S. et al. mtDNA Activates cGAS signaling and suppresses the YAP-Mediated endothelial cell proliferation program to promote inflammatory injury. *Immunity* **52**, 475–486 (2020).
109. Kondolf, H. C., D'Orlando, D. A., Dubyak, G. R. & Abbott, D. W. Protein engineering reveals that gasdermin A preferentially targets mitochondrial membranes over the plasma membrane during pyroptosis. *J. Biol. Chem.* **299**, 102908 (2023).

110. Zhang, F.-R. et al. Genomewide association study of leprosy. *N. Engl. J. Med.* **361**, 2609–2618 (2009).
111. Hui, K. Y. et al. Functional variants in the *LRRK2* gene confer shared effects on risk for Crohn's disease and Parkinson's disease. *Sci. Transl. Med.* **10**, eaai7795 (2018).
112. Weindel, C. G. et al. Mitochondrial ROS promotes susceptibility to infection via gasdermin D-mediated necroptosis. *Cell* **185**, 3214–3231 (2022).
113. Zhou, R., Yazdi, A. S., Menu, P. & Tschopp, J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* **469**, 221–225 (2011).
114. Bauernfeind, F. et al. Cutting Edge: reactive oxygen species inhibitors block priming, but not activation, of the NLRP3 Inflammasome. *J. Immunol.* **187**, 613–617 (2011).
115. Semino, C., Carta, S., Gattorno, M., Sitia, R. & Rubartelli, A. Progressive waves of IL-1β release by primary human monocytes via sequential activation of vesicular and gasdermin D-mediated secretory pathways. *Cell Death Dis.* **9**, 1088 (2018).
116. Wang, Y. et al. Mitochondrial ROS promote macrophage pyroptosis by inducing GSDMD oxidation. *J. Mol. Cell. Biol.* **11**, 1069–1082 (2019).
117. Bradfield, C. J. et al. Biphasic JNK signaling reveals distinct MAP3K complexes licensing inflammasome formation and pyroptosis. *Cell Death Differ.* **30**, 589–604 (2023).
118. Evavold, C. L. et al. Control of gasdermin D oligomerization and pyroptosis by the Ragulator–Rag–mTORC1 pathway. *Cell* **184**, 4495–4511 (2021).
119. Devant, P. et al. Gasdermin D pore-forming activity is redox-sensitive. *Cell Rep.* **42**, 112008 (2023).
120. Balasubramanian, A. et al. Palmitoylation of gasdermin D directs its membrane translocation and pore formation in pyroptosis. Preprint at *bioRxiv* https://doi.org/10.1101/2023.02.21.529402 (2023).
121. Du, G. et al. ROS-dependent palmitoylation is an obligate licensing modification for GSDMD pore formation. Preprint at *bioRxiv* https://doi.org/10.1101/2023.03.07.531538 (2023).
122. Johnson, A. G. et al. Bacterial gasdermins reveal an ancient mechanism of cell death. *Science* **375**, 221–225 (2022).
123. Hu, L. et al. Chemotherapy-induced pyroptosis is mediated by BAK/BAX–caspase-3–GSDME pathway and inhibited by 2-bromopalmitate. *Cell Death Dis.* **11**, 281 (2020).
124. Bambouskova, M. et al. Itaconate confers tolerance to late NLRP3 inflammasome activation. *Cell Rep.* **34**, 108756 (2021).
125. Hooftman, A. et al. The immunomodulatory metabolite itaconate modifies NLRP3 and inhibits inflammasome activation. *Cell Metab.* **32**, 468–478 (2020).
126. Humphries, F. et al. Succination inactivates gasdermin D and blocks pyroptosis. *Science* **369**, 1633–1637 (2020).
127. Hu, J. J. et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. *Nat. Immunol.* **21**, 736–745 (2020).
128. Rathkey, J. K. et al. Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis. *Sci. Immunol.* **3**, eaat2738 (2018).
129. Wang, C. et al. NLRP3 inflammasome activation triggers gasdermin D–independent inflammation. *Sci. Immunol.* **6**, eabj3859 (2021).
130. Li, Y., Pu, D., Huang, J., Zhang, Y. & Yin, H. Protein phosphatase 1 regulates phosphorylation of gasdermin D and pyroptosis. *Chem. Commun.* **58**, 11965–11968 (2022).
131. Hornbeck, P. V. et al. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. *Nucleic Acids Res.* **43**, D512–D520 (2015).
132. Shi, Y. et al. E3 ubiquitin ligase SYVN1 is a key positive regulator for GSDMD-mediated pyroptosis. *CellDeath Dis.* **13**, 106 (2022).
133. Luchetti, G. et al. Shigella ubiquitin ligase IpaH7.8 targets gasdermin D for degradation to prevent pyroptosis and enable infection. *Cell Host Microbe* **29**, 1521–1530(2021).
134. Hansen, J. M. et al. Pathogenic ubiquitination of GSDMB inhibits NK cell bactericidal functions. *Cell* **184**, 3178–3191 (2021).
135. Yin, H. et al. Insights into the GSDMB-mediated cellular lysis and its targeting by IpaH7.8. *Nat. Commun.* **14**, 61 (2023).
136. Chai, Q. et al. A bacterial phospholipid phosphatase inhibits host pyroptosis by hijacking ubiquitin. *Science* **378**, eabq0132 (2022).
137. Wein, T. & Sorek, R. Bacterial origins of human cell-autonomous innate immune mechanisms. *Nat. Rev. Immunol.* **22**, 629–638 (2022).
138. Daskalov, A. & Glass, N. L. Gasdermin and gasdermin-like pore-forming proteins in invertebrates, fungi and bacteria. *J. Mol. Biol.* **434**, 167273 (2022).
139. Angosto-Bazarra, D. et al. Evolutionary analyses of the gasdermin family suggest conserved roles in infection response despite loss of pore-forming functionality. *BMC Biol.* **20**, 9 (2022).
140. Jones, J. D. G., Vance, R. E. & Dangl, J. L. Intracellular innate immune surveillance devices in plants and animals. *Science* **354**, aaf6395 (2016).
141. Gao, L. A. et al. Prokaryotic innate immunity through pattern recognition of conserved viral proteins. *Science* **377**, eabm4096 (2022).
142. De Schutter, E. et al. Punching holes in cellular membranes: biology and evolution of gasdermins. *Trends Cell Biol.* **31**, 500–513 (2021).
143. Yuan, Z., Jiang, S., Qin, K. & Sun, L. New insights into the evolutionary dynamic and lineage divergence of gasdermin E in metazoa. *Front. Cell Dev. Biol.* **10**, 952015 (2022).
144. Jiang, S., Gu, H., Zhao, Y. & Sun, L. Teleost gasdermin E Is cleaved by caspase 1, 3 and 7 and induces pyroptosis. *J. Immunol.* **203**, 1369–1382 (2019).
145. Xu, H., Jiang, S., Yu, C., Yuan, Z. & Sun, L. GSDMEa-mediated pyroptosis is bi-directionally regulated by caspase and required for effective bacterial clearance in teleost. *Cell Death Dis.* **13**, 491 (2022).
146. Li, H. et al. Duck gasdermin E is a substrate of caspase-3/-7 and an executioner of pyroptosis. *Front. Immunol.* **13**, 078526 (2023).
147. Jiang, S., Zhou, Z., Sun, Y., Zhang, T. & Sun, L. Coral gasdermin triggers pyroptosis. *Sci. Immunol.* **5**, eabd2591 (2020).
148. Daskalov, A., Gladieux, P., Heller, J. & Glass, N. L. Programmed cell death in neurospora crassa is controlled by the allorecognition determinant rcd-1. *Genetics* **213**, 1387–1400 (2019).
149. Daskalov, A., Mitchell, P. S., Sandstrom, A., Vance, R. E. & Glass, N. L. Molecular characterization of a fungal gasdermin-like protein. *Proc. Natl Acad. Sci. USA* **117**, 18600–18607 (2020).
150. Clavé, C. et al. Fungal gasdermin-like proteins are controlled by proteolytic cleavage. *Proc. Natl Acad. Sci. USA* **119**, 2109418119 (2022).

Acknowledgements

We thank C. Evavold, as well as all members of the J.C.K. laboratory for helpful discussions. This work was supported by the National Institutes of Health grants AI167993, AI116550 and DK34854 to J.C.K. P.D. was supported by a scholarship by the Boehringer Ingelheim Fonds.

Author contributions

Conceptualization: P.D. and J.C.K.; visualization, P.D.; writing—original draft, P.D.; writing—review & editing, P.D. and J.C.K.; funding acquisition, P.D. and J.C.K.; supervision, J.C.K.

Competing interests

J.C.K. consults and holds equity in Corner Therapeutics, Larkspur Biosciences and Neumora Therapeutics. None of these relationships influenced this study. The other authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to Jonathan C. Kagan.

Peer review information *Nature Immunology* thanks Katherine Fitzgerald and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Ioana Visan, in collaboration with the rest of the *Nature Immunology* team.