Gastroenterology Report, 6(3), 2018, 167–176

doi: 10.1093/gastro/goy011
Advance Access Publication Date: 2 May 2018
Review

REVIEW

Inflammatory caspase-related pyroptosis: mechanism, regulation and therapeutic potential for inflammatory bowel disease

Yuan-Yuan Yuan[1], Ke-Xin Xie[2], Sha-Long Wang[1] and Lian-Wen Yuan[1,*]

[1]Department of Geriatric Surgery, The Second Xiangya Hospital of Central South University, Changsha, Hunan 410011, China and [2]Medical Laboratory Technology 1602, Central South University Xiangya School of Medicine, 172 Tongzipo Road, YueLu District, Changsha, Hunan 410011, China

*Corresponding author. Department of Geriatric Surgery, The Second Xiangya Hospital of Central South University, 139 Renming Zhong Road, Changsha, Hunan 410011, China. Tel: +86-13975198792; Email: yuanlianwen@csu.edu.cn

Abstract

As an essential part of programmed cell death, pyroptosis is an inflammatory response that is elicited upon infection by intracellular pathogens. Metabolic diseases, atherosclerosis and vital organ damage occur if pyroptosis is over-activated. Macrophages are the main cells that induce pyroptosis with the help of intracellular pattern-recognition receptors stimulated by danger signals and pathogenic microorganisms in the cytosol of host cells. Activated inflammatory caspases induce pyroptosis and produce pro-inflammatory cytokines, such as interleukin-1β and interleukin-18. Inflammatory programmed cell death is classified as canonical or non-canonical based on inflammatory caspases, which includes caspase-1 (in human and mouse) and caspase-11 (in mouse) or caspase-4 and -5 (in humans). Activated inflammatory caspases cleave the pore-forming effector protein, gasdermin-D, inducing osmotic pressure deregulation of internal fluids and subsequently rupturing the cell membranes. Inflammatory caspases could be attractive therapeutic targets for inflammatory bowel disease (IBD) in which pyroptosis may play an important role. This article reviews the current understanding of the mechanism of pyroptosis, focusing on the regulation of inflammatory caspases and therapeutic strategies for IBD.

Key words: Pyroptosis; gasdermin-D; inflammasome; caspase; inflammatory bowel disease

Introduction

Pyroptosis is a form of programmed cell death and plays a critical role in both tissue homeostasis and immune response. Dysregulation of pyroptosis promoted by microbial infection and danger signals is often associated with immune dysregulation and excessive inflammation response can result in a number of inflammatory diseases. Pyroptosis is characterized by macrophage cell membrane rupture brought about by cell swelling and lysis. Furthermore, these cells release pro-inflammatory cytokines and intracellular contents [1]. Pyroptosis was first presented as an unusual caspase-1-dependent mechanism of necrosis in macrophages infected with *Salmonella typhimurium*, and was different from traditional necrosis and apoptosis [2]. The newly caspase-1-dependent pro-inflammatory form of programmed cell death was named pyroptosis and arises from microbial infection and danger signals, including crystalline substances, monosodium urate, asbestos, silica crystals toxins and extracellular ATP [3, 4]. It is well known that pyroptosis plays a critical role in resisting

Submitted: 17 January 2018; **Revised:** 1 March 2018; **Accepted:** 18 March 2018

microbial colonization [5]. In addition, pyroptosis contributes to the pathogenesis of autoinflammatory and autoimmune diseases, such as kidney diseases [6], neurodegenerative diseases [7, 8], ischemia-reperfusion [9], liver fibrosis [10], colon cancer [11] and atherosclerosis [12].

Although different inflammatory caspases can cause canonical or non-canonical inflammasome pathway activation, the co-substrate gasdermin-D (GSDMD) causes them to have identical morphological changes. Pyroptosis forms pores in the plasma membrane using a necrotic N-terminal fragment [13]. A study of *Salmonella*-infected macrophages found that functional pyroptotic pores were around the 1.1–2.4-nm range in diameter based on the size of osmoprotectant molecules that prevented cell lysis [14, 15]. Recent studies suggest that DNFA5 is cleaved by caspase-3 into Asp270, which generates a necrotic DFNA5-N fragment that could induce secondary necrosis or pyroptosis by forming cell membrane pores [16, 17]. DNFA5 induces the signaling pathways of pyroptosis. The relationship between pyroptosis and other gasdermin family members, including GSDMA, GSDMB and GSDMA3, on the ability to form gasdermin N-terminal domains by specific inducers needs to be deciphered. Caspase-3, a distinctive apoptotic caspase, may play an important role between apoptosis and pyroptosis.

Inflammatory bowel disease (IBD) is a chronic, relapsing and incurable disease of the intestinal tract, which includes Crohn's disease (CD) and ulcerative colitis (UC). Although research has shown that the pathogenesis of IBD is closely related to mucosal barrier damage, dysbacteriosis and immunity dysregulation, the mechanisms underlining the pathogenesis have not been fully understood [18]. There is increasing evidence that pattern-recognition receptors may play an important role in maintaining immune-system homeostasis, modulating the gastrointestinal microflora and facilitating intestinal epithelium regeneration and repair [19]. Numerous studies have indicated the role of NOD-like receptors (NLRs) in IBD on forming signaling complexes called inflammasomes. These complexes cleave and activate pro-cytokines interleukin-1β (IL-1β) and IL-18 into mature IL-1β and IL-18, to induce pyroptosis [20].

In this review, we aim to explore of the regulatory mechanism of pyroptosis and inflammatory caspases in immune dysregulation. Of particular importance is the therapeutic potential for IBD through targeting pyroptosis signaling.

Regulation of inflammatory caspases in pyroptosis

Caspases are a family of highly conserved aspartate-specific cysteines with 15 mammalian members. The majority can be grouped into inflammatory caspases and apoptotic caspases [21]. The former includes caspase-1, -4, -5 and -11, and the latter consists of caspase-2, -3, -6, -7, -8, -9 and -10 [22]. The initiation of pyroptosis and apoptosis relies on specific caspases to induce their respective programmed cell death pathways [23]. Unlike apoptotic caspases, inflammatory caspases are not categorized as initiators or effectors, but induce a form of necrotic or inflammatory programmed cell death, namely pyroptosis [24]. Previous studies have characterized the various contributions of inflammatory caspases in murine models of salmonellosis [25]. Pyroptosis was first described as a mediator of innate immune responses in macrophages and is initiated by caspase-1, resulting in the secretion of IL-1β and IL-18 [2, 14]. IL-1β is an inducer of vasodilation, immune-cell extravasation and inflammation [26]. It also has a significant role in shaping adaptive immunity [26]. IL-18 induces interferon (IFN)-γ production in Th1 cells, NK cells and cytotoxic T cells, participates in Th2 development and enhances local inflammation [27]. Subsequent studies have demonstrated that pyroptosis also participates in eliminating other bacterial infections, not just *Salmonella*, even in the absence of IL-1β and IL-18 [5].

Studies have demonstrated that all inflammatory caspases can be activated by the inflammasome [28, 29]. Caspases associate with inflammasome-initiating sensors (NLRP1, NLRP3, NLRC4, AIM2 or pyrin) to form macromolecular protein complexes even in the absence of the inflammasome adapter protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) [28, 30]. Necroptosis, defined as a necrotic and inflammatory form of programmed cell death, in the absence of caspase signaling, can be distinguished from pyroptosis [31, 32]. Pyroptosis can be induced by different inflammatory caspases and presents morphological characteristics such as cell swelling, positivity for Annexin V and TUNEL staining, chromatin condensation and membrane pore formation, but with the absence of DNA laddering [14, 15, 33]. Consequently, the cells rupture and release cytoplasmic contents, pro-inflammatory cytokines, endogenous ligands, alarmins and other toxins [34–37].

In the following sections, the canonical and non-canonical inflammasome pathway and the two major pyroptosis signaling pathways will be discussed. The canonical inflammasome pathway is activated by caspase-1 and assembled by cytoplasmic sensors, such as NLRs. The non-canonical inflammasome pathway is related to caspase-4/5/11 and is stimulated by immune-system activators, such as lipopolysaccharide (LPS), which are the components of the bacterial cell wall [38, 39].

Canonical inflammasome pathway in pyroptosis

Depending on the inflammation-initiating sensors to recognize pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs), caspase-1 activation can induce the canonical inflammation pathway in pyroptosis [31, 40]. The C-terminal caspase recruitment domain is considered the 'bridge' between pro-caspase-1 and NLRs containing pyrin domains. ASC has an important role in multiple inflammatory diseases and autoimmune diseases associated with inflammasome dysfunction [41–43]. As mentioned previously, Pyrin, Aim2 and NLRs have been associated with pyroptosis. NLR is one of the inflammation-initiating sensors that consists of a tripartite structure: a C-terminal leucine-rich repeat that detects PAMP; a central nucleotide-binding oligomerization domain that mediates self-oligomerization important for activation; and an N-terminal effector domain, which acts as an N-terminal caspase recruitment domain, pyrin domain or baculovirus inhibitor repeat [44]. Inflammation-initiating sensors commit themselves to recognizing danger signals, such as pathogens, foreign bodies and host molecules [45, 46]. They invade the host cell cytoplasm and trigger the activation of caspase-1 [45, 46], which leads to inflammation assembly and inflammatory cytokines IL-18 and IL-1β secretion.

Although there are slight differences in the activation modes among the different microbial infections, a series of experiments have demonstrated similar molecular mechanisms. For instance, inflammasome sensors of NLRP1b and NLRP1 in mouse are activated by Bacillus anthracis poisons [47]. AIM2 is

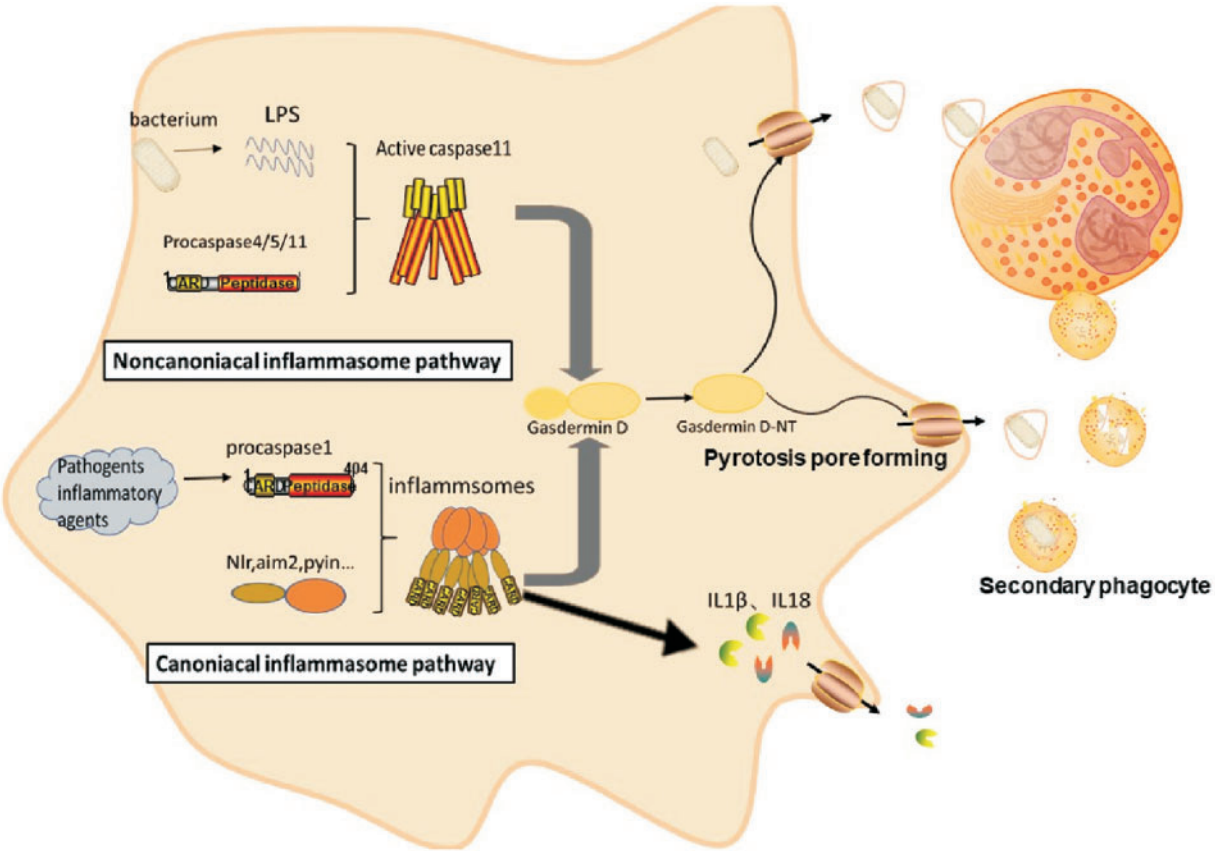

Figure 1. Inflammatory caspases (caspase-1 and caspase-4/5/11) are activated through canonical or non-canonical inflammasomes, triggered by microorganism infection. In the non-canonical pathway, procaspase-4/5/11 directly binds to lipopolysaccharide (LPS), resulting in their activation. In the canonical pathway, inflammasome-initiating sensors (NLRP1, NLRP3, NLRC4, AIM2 or pyrin) recognize pathogens and danger signals, and form multimerization of inflammasomes binding with caspase-1. Upon activation, inflammatory caspases cleave gasdermin-D to produce gasdermin-D N-terminal (NT) fragments. Gasdermin-D NT as a pore-forming protein contributes to pyroptosis pore-forming in the infected cell. This then releases cytokines and debris of pyroptotic macrophages, which had previously trapped intracellular bacteria to further promote uptake into neutrophils by efferocytosis (secondary phagocytes).

activated by direct binding to the cytoplasmic dsDNA [48]. NLRP3 is activated primarily by cytosolic flagellin and the NEK7 kinase has recently been demonstrated to play a role in upstream signaling [49, 50]. Pyrin responds to bacterial toxin-induced modifications of Rho GTPases and requires microtubule assembly for its activation [51]. Unlike other inflammation-initiating sensors, NLRC4 does not have an N-terminal pyrin domain and binds directly to the inner rod, flagellin or needle proteins of the Type III secretion system of bacteria, which then activates pyroptosis accompanied by the secretion of IL-1β [52]. Conversely, abundant IL-1β would increase ASC speck formation and synergize with cofactors to promote pattern recognition to assist NLRC4 signaling [53]. Increasing evidence suggests that interactions between multiple NLRs may contribute to inflammasome formation [1].

Host cells interact with caspase-1-activating ligands and sensors to trigger common downstream signaling to induce pyroptosis [54]. In addition, the adapter protein ASC can self-associate and form analogous complexes without an NLR to form inflammasomes [5]. For instance, *Salmonella*-mediated caspase-1 activation is downregulated in ASC-deficient macrophages, suggesting that NLRC4 facilitates caspase-1 activation, rather than completely relying on ASC [52]. In general, caspase-1 can be activated by different inflammation-initiating sensors mentioned above, such as inflammasomes, which are the cytosolic multiprotein complexes assembled by intracellular nucleotide-binding oligomerization NLRs. Caspase-1 induces pro-inflammatory cytokines (pro-IL-1β, pro-IL-18) and gasdermin-D N-terminal domains to form pores in the plasma membrane of an infected cell (details discussed below), which then causes infected cells to release bacteria and inflammatory cytokines (Figure 1).

Non-canonical inflammasome pathway in pyroptosis

Caspase-1-independent pyroptosis can be activated by non-canonical inflammasomes—to be precise, by human caspase-4, human caspase-5 or mouse caspase-11 [55]. Unlike the canonical inflammasome pathway, these caspases recognize LPS from gram-negative bacteria with caspase recruitment domains in the host cytoplasm [39, 56]. A previous study demonstrated that Casp1-/-Casp11-/- mice with orogastric infection caused by *S. typhimurium* have more bacteria in their systemic organs compared to Casp1-/-Casp11Tg mice, while both strains of mice have more bacteria than wild-type mice [57]. This suggests that caspase-11 increases susceptibility to *Salmonella* infection. Moreover, intracellular *Salmonella* could induce host cell death via caspase-11 in the absence of caspase-1 [57, 58]. Knodler *et al.* [59] demonstrated that IL-18 secretion in intestinal tissue in

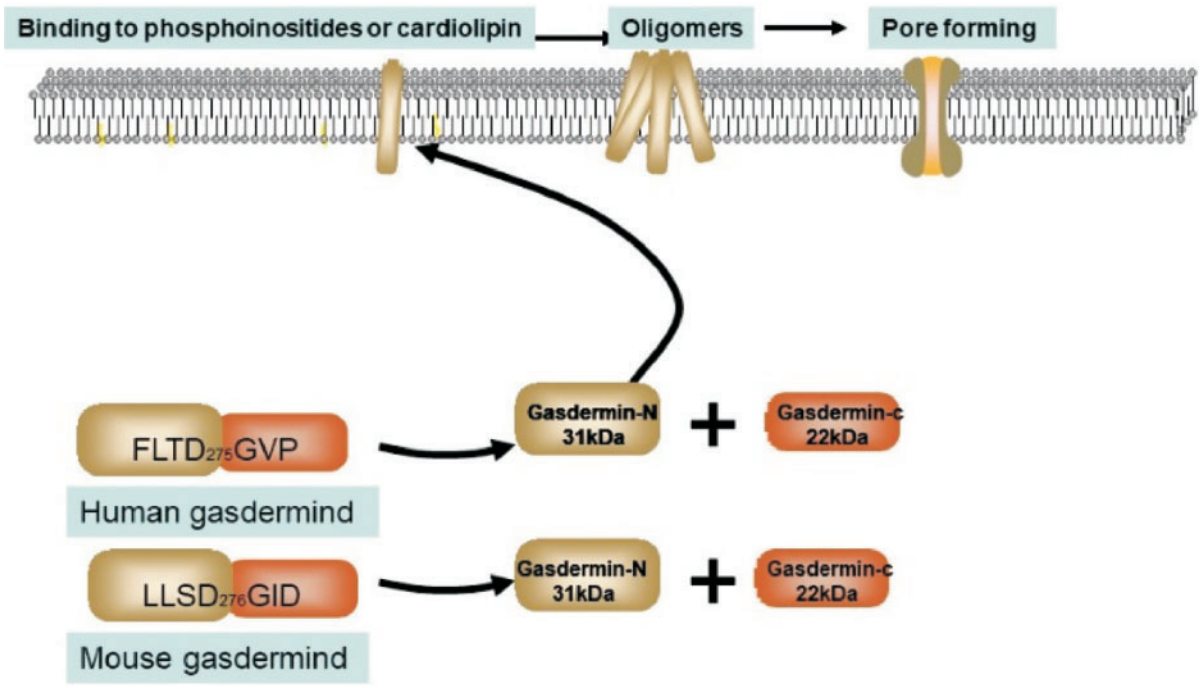

Figure 2. Gasdermin-D, containing 276GVPAEG281 or 272FLTD275 in human and 273LLSD276 in mouse, is cleaved by caspase-1, -4, -5 or -11. The cleavage products result in a 31-kDa product of gasdermin-D. The activated Gasdermin-D N-terminal domain (31 kDa) then binds to phosphoinositides or cardiolipin in the plasma membrane to form multiple monomers that oligomerize to form a gasdermin pore.

response to *S. typhimurium* infection was dependent on caspase-11 to control bacterial numbers in the cecum. Kayagki *et al.* [60] showed that caspase-11 can trigger pyroptosis, although weaker in directly cleaving pro-IL-1β and pro-IL-18, and Knodler *et al.* [59] demonstrated that caspase-4 has the ability to cleave pro-IL-1β and pro-IL-18. Numerous pieces of research have suggested that non-canonical inflammasome pathways play a dominant role during acute inflammation, whereby activation of caspase-11 brings about IL-1β and IL-18 cleavage, which is a typical mechanism of the canonical inflammasome pathway [61–63] (Figure 1).

GSDMD N-terminal domains induce pyroptosis

GSDMD is a common substrate for inflammatory caspases and has recently been determined to be the mechanistic link between caspase signaling and pyroptosis [60, 64, 65]. Numerous studies have demonstrated that the N-terminal fragment of GSDMD is the key player in inducing pyroptosis, not merely *in vitro*, but also *in vivo*. In addition, only in the presence of both GSDMD and caspase-1 were liposomes able to release dye, suggesting membrane pore formation [15, 40, 60, 66]. Additional studies have demonstrated that GSDMD was cleaved by caspase-1, 4, 5 or 11, but not with apoptotic caspases (caspase-2, -8 and -9) in 293 T cells. The cleavage products (31 kDa) of GSDMD contain 276GVPAEG281 or 272FLTD275 in human and 273LLSD276 in mouse [67]. The cleavage of GSDMD is required for pyroptosis. The D/A mutant of GSDMD (Asp276 in mouse gasdermin-D converted to A) is resistant to cleavage by caspase-1 and caspase-4/5/11 [67]. When these were overexpressed in 293 T cells, they were unable to induce pyroptosis [67]. A current hypothesis on the mechanism by which GSDMD induces pyroptosis is that the GSDMD N-terminal domain binds to phosphoinositides and cardiolipin, then oligomerizes to form pores on the bacterial cell membrane [64, 66] (Figure 2). Specifically, cleaved GSDMD is found to selectively bind to plasma membranes containing lipids, such as the mitochondrial and bacterial lipid cardiolipin, and phosphatidylinositol phosphates that are located on the inner leaflet of the mammalian cell membrane [64]. Binding results in the formation of oligomeric pores that subsequently kill mammalian cells, which is restricted to activated cells and does not kill bystander cells [64]. However, the relationship between GSDMD and secretion of IL-1β and IL-18 remains to be elucidated.

Gasdermin-D: a direct executor of the canonical inflammatory pathway

In the past two years, researchers have demonstrated that GSDMD functioned downstream of inflammatory caspases [13, 68–70] and formed large permeability circular pores with ring diameters around 20 nm [69]. Several studies showed that caspase-1-mediated pyroptosis in iBMDM cells was induced by the very potent agonist of NAIP–NLRC4 inflammasome, namely *Burkholderia thailandensis* (LFn–BsaK), while GSDMD-/- iBMDM cells were resistant [40, 71, 72]. That suggests that GSDMD plays a critical role in caspase-1-mediated pyroptosis downstream of the NAIP–NLRC4 inflammasome. The N-terminal fragment of GSDMD can also drive the activation of the NLRP3-dependent caspase-1 inflammasome [40, 60, 73], possibly requiring potassium efflux caused by GSDMD-induced membrane pores [74]. GSDMD-mediated pyroptosis plays an important role in mature IL-1β release but does not affect its maturation [65]. In summary, caspase-1-mediated pyroptosis is induced by GSDMD, which may or may not involve the secretion of cytokines in innate immunity.

Gasdermin-D: a direct executor of the non-canonical inflammatory pathway

Yang *et al.* [71] and Zhao *et al.* [72] performed an unbiased genome-wide genetic screen using CRISPR-Cas9 technology to identify new components in LPS/caspase-4/11-induced pyroptosis. Similarly to mice lacking caspase-11, mice lacking GSDMD were remarkably resistant to LPS-induced endotoxemia compared to wild-type mice [60]. The study found that GSDMD-/- iBMDM cells regained the sensitivity to LPS electroporation when complemented with either human or mouse GSDMD. Both caspase-11 and caspase-4/5 were activated by direct binding of LPS to trigger pyropotosis via GSDMD, which would be the likely mechanism against cytosolic-invasive gram-negative bacteria.

Pyroprosis triggers pore-induced intracellular trap (PIT) and neutrophil extracellular trap (PET)

Viable bacteria can remain trapped within the cellular debris of pyroptotic macrophages, termed PITs. Jorgensen *et al.* [75] showed that pyroptosis had specific immunological functions to further promote uptake into neutrophils by efferocytosis *in vivo*. A more popular view is that both unbound and trapped bacteria are released by pyroptotic cells, which can be captured by phagocytic cells in tissues [31]. An advantage of the capture of pyroptosis-released bacteria by neutrophils is that these host cells are relatively resistant to pyroptosis in response to *S. typhimurium* infection and other inflammasome activators [76]. Pyroptosis can trigger the release of intracellular bacteria residing in macrophages, including *S. typhimurium*, *Legionella pneumophila* and *Burkholderia thailandensis*. These newly released bacteria can then be phagocytosed and killed by neutrophils via a mechanism dependent on the production of reactive oxygen species but independent of IL-1β and IL-18 (Figure 1). In mice that lack NADPH oxidase NOX2, neutrophils can undergo pyroptosis infected with *Pseudomonas aeruginosa*, suggesting that pyroptosis can be activated to compensate for lack of anti-microbial pathway activity [77]. NETs and PITs act independently of each other, as NET-defective mice (Mpo−/− and Elane−/−) are fully competent to clear bacteria trapped in PITs, and NET is not inhibited by the pan-caspase inhibitor z-VAD-fmk [75, 77–79].

The role of the gasdermin-family in pyroptosis

DFNA5 was discovered as a physiological substrate for caspase-3 and the N-terminal domain of DFNA5, which induces cell death by attacking the plasma membrane [16, 17, 66]. Similar pathways were observed in other gasdermin family members, including GSDMA, GSDMB, GSDMC, DFNA5 and DFNB59 [16, 66]. N-terminal domains of the gasdermin family, but not the full-length proteins or N-terminal domains, are efficiently and specifically precipitated by cardiolipin liposomes [66]. Whether other gasdermin family members exacerbate programmed cell death or mediate different cell death pathways is currently being investigated. Several diseases have been linked to gasdermin and whether pyroptosis plays an important role in these diseases requires further study (Table 1). Chao *et al.* [82] showed that the crystal structure of the GSDMB N-terminal domain was linked to IBD. Interestingly, caspase-3, -6 and -7, rather than the inflammatory caspases, cleaved GSDMB [40]. Rogers *et al.* [16] demonstrated that caspase3 and GSDMD together induced apoptosis, rather than pyroptosis, but pyroptosis could be induced when GSDMD was substituted for GSDME. Apoptosis mediated by apoptotic caspases (such as caspase-2, -3, -6, -7, -8 and -9 [87]), results in poly (ADP-ribose) polymerase and inhibitor of caspase-activated DNase to undergo proteolysis to cleave DNA into oligo nucleosomal DNA fragments [14]. This pathway differs from pyroptosis, although DNA cleavage could sometimes be triggered [14, 88]. Apoptosis has generally been considered an immunologically silent process, although emerging evidence indicates that apoptosis can be inflammatory when induced under certain circumstances and has roles in host defense against infection [89–91]. There may be possible interplays between apoptosis and pyroptosis.

Pyroptosis of intestinal epithelial cells in IBD

With the expanding urbanization of newly industrialized countries, such as India and China, IBD, ‘ a Western disease’, is gradually growing into a global disease [92, 93]. Although the use of glucocorticoids, immunosuppressants, biological reagents and antibiotics reduces the symptoms of IBD, a minority of patients will eventually need surgical treatment. This has led to a considerable rise in health-care costs over time [94–97]. Prevention, diagnosis and effective therapy are the challenges. IBD is one of the most common abnormal inflammation diseases of the gastrointestinal tract. Intestinal epithelial cells (IEC) serve as a barrier between the host cell and the intestinal microbiota [98]. Excessive inflammasome expression in IEC results in an imbalance between the immune system and gut microbiota. Immune treatment strategies targeting pyroptosis in IEC of IBD may provide an alternative treatment and thereby reduce suffering and health-care costs. System homeostasis is maintained by pattern-recognition receptors (PRRs) [98, 99] and NLRs are important components that are dysregulated in IBD patients [100]. NLR inflammasomes are macromolecular platforms that sense cytosolic PAMPs and DAMPs, resulting in the maturation of IL-1β and IL-18, which is similar to the process of pyroptosis, as mentioned previously. A great deal of interest on whether or not pyroptosis plays a pivotal role in the development of IBD is currently being investigated.

Dextran sulfate sodium (DSS) mouse (ASC-/-) colitis models have a more severe phenotype than NLR knockout colitis mice [101–104], implying that loss of all inflammasomes is more detrimental to IBD pathogenesis than the loss of any individual sensor. NLR inflammasomes can attenuate gastrointestinal inflammation during experimental colitis [19, 105]. In addition, several studies have shown a significant increase in morbidity and mortality, weight loss, rectal bleeding and other pathological features of disease progression in mice lacking caspase-1 [101, 106, 107]. A series of experiments have shown that over-activation of inflammasome-initiating sensors results in intestine damage and intestinal inflammation. Strong upregulation of AIM2 and IFI16 inflammasomes in the mucosa of patients with active IBD [108] and A20-deficiency in macrophages significantly enhancing Nlrp3 inflammasome-mediated caspase-1 activation [109] demonstrates that the canonical inflammasome pathway and likely resulting over-activation of pyropotosis play a pivotal role in generation, progression and prognosis of IBD.

Similarly to the caspase-1 studies, caspase-11 has also been shown to attenuate gastrointestinal inflammation during experimental colitis in mice [103]. Research has demonstrated that caspase-11-/- mice appear to have more severe inflammation and epithelial cell damage in the colon than wild-type mice [103]. Caspase-11-/- mice were highly sensitive to acute DSS,

Table 1. Function of gasdermin family members

Gene name		Domains	Induces extensive pyroptosis	Activates caspases	Associated disease	References
Human	Mouse					
GSDMA (gasdermin 1)	Gsdma1-3	Gasdermin N- and C-terminal domains	Yes	Unknown	Aalopecia and excoriation	[80, 81]
GSDMB (gasdermin-like /GSDML)	Gsdmc1-4	Gasdermin N- and C-terminal domains	Yes	Caspase-3/6/7	Asthma and inflammatory bowel disease	[82, 83]
GSDMD (DFNA5L)	Gsdmd	Gasdermin N- and C-termianl domains	Yes	Caspase-1/4/5/11	Infection diseases and sepsis	[31, 84]
GSDME (DFNA5)	Dfna5	Gasdermin N- and C-terminal domains	Yes	Caspase-3	Autosomal-dominant congenital deafness	[17]
DFNB59 (pejvakin)	Dfnb59	Gasdermin N-terminal domain	No	Unknown	Autosomal-recessive congenital deafness	[85, 86]

independently of microbiome shifts [108]. However, the protective effects of caspase-11 appears to be only restricted to acute experimental colitis, as a lack of caspase-11 had only a minimal impact in chronic DSS and AOM + DSS models [103]. A similar phenomenon during canonical inflammasome pathway happens on non-canonical inflammasome pathway. In humans, the caspase-11 orthologues caspase-4 and caspase-5 were dramatically up-regulated in both CD and UC patient samples [109]. Further experiments revealed that caspase-1/5 expression was significantly higher in inflamed colonic but not ileal tissues. In summary, caspase-4/5/11 in the non-canonical inflammasome pathway may influence the development of inflammatory bowel disease [110] and pyroptosis induced by non-canonical inflammasomes in IBD should not be overlooked.

Pyroptosis presumably occurs in IBD, which is based on IEC interactions with immune cells located in the intestinal epithelium to affect cellular communication [111]. Inflammasomes, especially NLRP3, activated by inflammatory caspases play important roles in pyroptosis of IEC [50, 112–114]. Several lines of evidence have demonstrated that deficiencies in NLRP3 inflammasome components can protect mice from DSS-induced colitis [46, 115, 116]. The interaction between IEC integrin receptors and bacterial adhesin invasin provides the first signal for NLRP3 inflammasome activation in response to *Yersinia enterocolitica* infection, which is followed by the release of IL-18 and inflammatory responses at mucosal sites [117]. IL-1β secretion is not present in macrophages lacking NLRP3 and pharmacological inhibition of caspase-1 with pralnacasan can achieve a level of mucosal protection comparable to NLRP3 deficiency, indicating that DSS activates caspase-1 via the NLRP3 inflammasome pathway [118] (Figure 3). Other disease models of IBD also support the important role of NLRP3 in enteritis [119, 120] (Table 2).

In colonic macrophages, enterobacteriaceae induce intestinal inflammation via release of IL-1β mediated by NLRP3, which is similar to the pathogenic mechanism of *Salmonella* [125]. Our experiments demonstrated that the expression of caspase-1, NLRP3 and GSDMD was significantly higher in human inflammatory intestine tissues compared to normal intestine tissue (unpublished data). Schmid-Burgk *et al.* [49] found that macrophages with NLRP3 inflammasome activation would be rescued by targeting Nek7, which was thought to be upstream of Nlrp3 [126]. This study raises the hypothesis that Nek7 may be a key therapeutic target for pyroptosis. Inhibition of pyroptosis provides a potential therapeutic avenue to cure intestinal inflammation. Liu *et al.* [83] demonstrated that cholecalciterol cholesterol emulsion (CCE) was able to

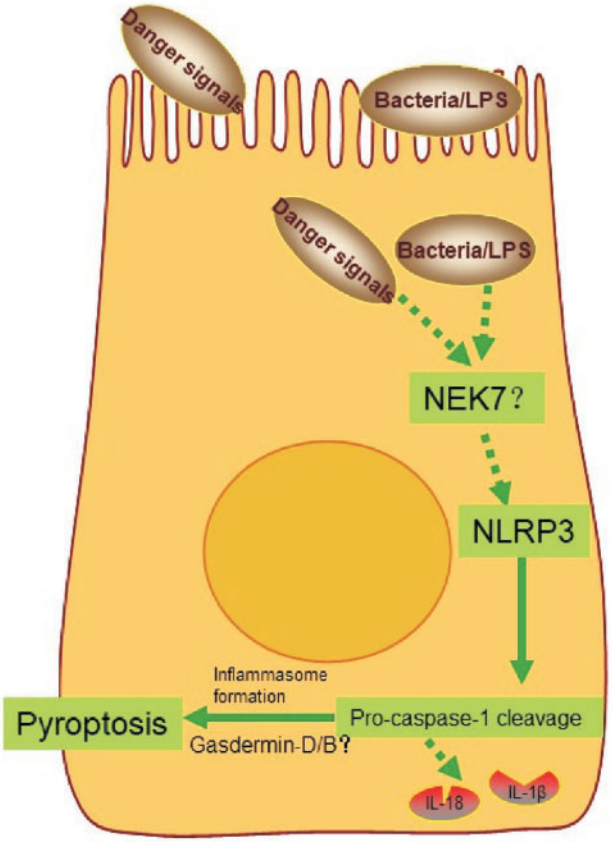

Figure 3. NLRP3 inflammasome activation is induced by bacteria and danger signals in intestinal epithelial cells (IEC). Nek7 is thought to be upstream of Nlrp3 but detailed mechanisms are unclear. Activated NLRP3 binds with cysteine protease caspase-1 to form a macromolecular complex, which directly cleaves gasdermin-D/B to initiate pyroptosis and inflammation. In addition, caspase-1 functions in the maturation and release of IL-1*β* and IL-18.

attenuate the degree of colitis in rat models by inhibiting pyroptosis, and observed the decrease of inflammatory responses (ASC, caspase-1, IL-1β, IL-18 and IL-6). CCE also provides an alternative therapeutic target for IBD patients and clinical trials have demonstrated its potential [83]. IEC infected with *S. typhimurium* undergo activation of the caspase-1 or caspase-11 inflammasome, resulting in physical entry of infected enterocytes from the intestine [119, 120, 127]. Hence, elimination of infected cells from tissues would be another mechanism by which pyroptosis eliminates

Table 2. NLRP3 in disease models of inflammatory bowel disease

	Pathogenic factors	Caspases	Cytokines	References
NLRP3	Clostridium	Caspase-1	IL-1β	[119]
	Helicobacter pylori		IL-18	[121]
	Mycobacterium		IL-1	[120]
	Enterobacteriaceae		IL-1β	[122]
	2, 4, 6-trinitrobenzene sulfonic acid (TNBS)		IL-18, IL-1β	[123]
	Dextran sulfate sodium (DSS)		IL-18, IL-1β	[123]
	Bacterial muramyl dipeptide		–	[124]

invasive intestinal bacteria. Davis *et al.* [20] suggested that inhibition of IEC pyroptosis may be the therapeutic mechanism of action of mesalamine and corticosteroids.

Conclusions and future directions

Pyroptosis is a form of programmed cell death and takes place in monocytes, macrophages and epithelial cells. The typical catalytic action of inflammatory caspases (caspase-1/4/5/11) during pyroptosis differs from that of apoptosis, and the morphological characteristics of membrane rupture are also different [128]. Despite the morphological features being similar to necroptosis, pyroptosis has its own characteristics of inducing a specific size of membrane pores and does not depend on receptor interacting protein (RIP) 1 and 3 signaling pathway for its actions [129, 130]. Pyroptosis depends on GSDMD to form specifically sized pores on the cell membrane, although the exact mechanism on how it does this has not been fully elucidated [66]. It is speculated that inflammatory caspases and GSDMD play important roles in pyroptosis. Future studies to find inhibitors of the pyroptosis signaling pathway may provide therapeutic potential for relevant diseases induced by pyroptosis.

The etiology and pathogenesis of IBD still need to be fully characterized. However, numerous studies have demonstrated that alteration of intestinal flora affects the intestinal mucosal immunity and leads to the occurrence of IBD [131]. In various models of IBD, inflammatory caspases were found to be significantly elevated, suggesting that pyroptosis may be involved in the development of IBD [110, 132]. Several studies have demonstrated the existence of pyroptosis in IBD. Xiong *et al.* [133] demonstrated that CCE could attenuate experimental colitis by suppressing pyroptosis signaling. Therapeutic drugs for IBD such as mesalamine and corticosteroids may relate to the inhibition of pyroptosis in IEC [20]. The signaling mechanism of pyroptosis has yet to be fully deciphered when compared to apoptosis. In addition, whether pyroptosis plays a significant role in IBD is still unclear. Further research into pyroptosis signaling pathways is necessary to support its therapeutic target for IBD.

Conflict of interest statement: none declared.

References

1. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* 2009;**7**:99–109.
2. Brennan MA, Cookson BT. Salmonella induces macrophage death by caspase-1-dependent necrosis. *Mol Microbiol* 2000; **38**:31–40.
3. Cookson BT, Brennan MA. Pro-inflammatory programmed cell death. *Trends Microbiol* 2001;**9**:113–4.
4. Lamkanfi M, Dixit V. Mechanisms and functions of inflammasomes. *Cell* 2014;**157**:1013–22.
5. Miao EA, Leaf IA, Treuting PM *et al.* Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nat Immunol* 2010;**11**:1136–42.
6. Darisipudi MN, Knauf F. An update on the role of the inflammasomes in the pathogenesis of kidney diseases. *Pediatr Nephrol* 2016;**31**:535–44.
7. Tan CC, Zhang JG, Tan MS *et al.* NLRP1 inflammasome is activated in patients with medial temporal lobe epilepsy and contributes to neuronal pyroptosis in amygdala kindling-induced rat model. *J Neuroinflammation* 2015;**12**:18.
8. Kovarova M, Hesker PR, Jania L *et al.* NLRP1-dependent pyroptosis leads to acute lung injury and morbidity in mice. *J Immunol* 2012;**189**:2006–16.
9. Yang JR, Yao FH, Zhang JG *et al.* Ischemia-reperfusion induces renal tubule pyroptosis via the CHOP-caspase-11 pathway. *Am J Physiol Renal Physiol* 2014;**306**:F75–84.
10. Wree A, Eguchi A, Mcgeough MD *et al.* NLRP3 inflammasome activation results in hepatocyte pyroptosis, liver inflammation, and fibrosis in mice. *Hepatology* 2014;**59**:898–910.
11. Derangère V, Chevriaux A, Courtaut F *et al.* Liver X receptor *β* activation induces pyroptosis of human and murine colon cancer cells. *Cell Death Differ* 2014;**21**:1914–24.
12. Chang W, Lin J, Dong J *et al.* Pyroptosis: an inflammatory cell death implicates in atherosclerosis. *Med Hypotheses* 2013;**81**: 484–6.
13. Shi J, Gao W, Feng S. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci* 2017;**42**: 245–54.
14. Fink SL, Cookson BT. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell Microbiol* 2006;**8**:1812–25.
15. Aglietti RA, Estevez A, Gupta A *et al.* GsdmD p30 elicited by caspase-11 during pyroptosis forms pores in membranes. *Proc Natl Acad Sci U S A* 2016;**113**:7858–63.
16. Rogers C, Fernandes-Alnemri T, Mayes L *et al.* Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. *Nat Commun* 2017;**8**:14128.
17. Wang Y, Gao W, Shi X *et al.* Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a Gasdermin. *Nature* 2017;**547**:99–103.
18. Sobolewska-Włodarczyk A, Włodarczyk M. *Pathogenesis of IBD*. Berlin, German: Springer International Publishing, 2017.
19. Ringel-Scaia VM, Mcdaniel DK, Allen IC. The goldilocks conundrum: NLR inflammasome modulation of gastrointestinal inflammation during inflammatory bowel disease. *Crit Rev Immunol* 2016;**36**:283–314.
20. Davis EM, Kaufmann Y, Goyne H *et al.* Pyroptosis of intestinal epithelial cells is crucial to the development of mucosal

barrier dysfunction and intestinal inflammation. *Gastroenterology* 2017;**152**:S967.
21. Chowdhury I, Tharakan B, Bhat GK. Caspases—an update. *Comp Biochem Physiol B Biochem Mol Biol* 2008;**151**:10–27.
22. Siegel RM. Caspases at the crossroads of immune-cell life and death. *Nat Rev Immunol* 2006;**6**:308–17.
23. Miao EA, Rajan JV, Aderem A. Caspase-1-induced pyroptotic cell death. *Immunol Rev* 2011;**243**:206–14.
24. Vande Walle L, Lamkanfi M. Pyroptosis. *Curr Biol* 2016;**26**: R568–72.
25. Franchi L, Amer A, Body-Malapel M *et al*. Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages. *Nat Immunol* 2006;**7**:576–82.
26. Joosten LA, Netea MG, Dinarello CA. Interleukin-1β in innate inflammation, autophagy and immunity. *Semin Immunol* 2013;**25**:416–24.
27. Novick D, Kim SH, Fantuzzi G *et al*. Interleukin18 binding protein: a novel modulator of the Th1 cytokine response. *Immunity* 1999;**10**:127–36.
28. Man SM, Kanneganti TD. Regulation of inflammasome activation. *Immunol Rev* 2015;**265**:6–21.
29. Rathinam VA, Fitzgerald KA. Inflammasome complexes: emerging mechanisms and effector functions. *Cell* 2016;**165**: 792–800.
30. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol Cell* 2002;**10**:417–26.
31. Liu X, Lieberman J. A mechanistic understanding of pyroptosis: the fiery death triggered by invasive infection. *Adv Immunol* 2017;**135**:81–117.
32. Weinlich R, Oberst A, Beere HM *et al*. Necroptosis in development, inflammation and disease. *Nat Rev Mol Cell Biol* 2017; **18**:127–36.
33. Wang BX, Yin BL, He B *et al*. Overexpression of DNA damage-induced 45 α gene contributes to esophageal squamous cell cancer by promoter hypomethylation. *J Exp Clin Cancer Res* 2012;**31**:11.
34. Mariathasan S, Newton K, Monack DM *et al*. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 2004;**430**:213–8.
35. England H, Summersgill HR, Edye ME *et al*. Release of interleukin-1alpha or interleukin-1beta depends on mechanism of cell death. *J Biol Chem* 2014;**289**:15942–50.
36. Dombrowski Y, Peric M, Koglin S *et al*. Cytosolic DNA triggers inflammasome activation in keratinocytes in psoriatic lesions. *Sci Transl Med* 2011;**3**:82ra38.
37. Chen CJ, Kono H, Golenbock D *et al*. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat Med* 2007;**13**:851–6.
38. Broz P. Immunology: caspase target drives pyroptosis. *Nature* 2015;**526**:642–3.
39. Shi J, Zhao Y, Wang Y *et al*. Inflammatory caspases are innate immune receptors for intracellular LPS. *Nature* 2014; **514**:187–92.
40. Shi J, Zhao Y, Wang K *et al*. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 2015; **526**:660–5.
41. Yang CA, Chiang BL. Inflammasomes and human autoimmunity: a comprehensive review. *J Autoimmun* 2015;**61**:1–8.
42. Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* 2011;**29**:707–35.
43. Compan V, Martín-Sánchez F, Baroja-Mazo A *et al*. Apoptosis-associated speck-like protein containing CARD forms specks but does not activate caspase-1 in the absence of NLRP3 during macrophage swelling. *J Immunol* 2015;**194**: 1261–73.
44. Chen G, Shaw MH, Kim YG *et al*. NOD-like receptors: role in innate immunity and inflammatory disease. *Annu Rev Pathol* 2009;**4**:365–98.
45. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ* 2007;**14**:10–22.
46. Chen GY, Núñez G. Inflammasomes in intestinal inflammation and cancer. *Gastroenterology* 2011;**141**:1986–99.
47. Hellmich KA, Levinsohn JL, Fattah R *et al*. Anthrax lethal factor cleaves mouse nlrp1b in both toxin-sensitive and toxin-resistant macrophages. *PLoS One* 2012;**7**:e49741.
48. Bürckstümmer T, Baumann C, Blüml S *et al*. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol* 2009;**10**:266–72.
49. Schmid-Burgk JL, Chauhan D, Schmidt T *et al*. A genome-wide CRISPR screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. *J Biol Chem* 2016; **291**:103–9.
50. Shi H, Wang Y, Li X *et al*. NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat Immunol* 2015;**17**:250–8.
51. Gavrilin MA, Abdelaziz DH, Mostafa M *et al*. Activation of the pyrin inflammasome by intracellular Burkholderia cenocepacia. *J Immunol* 2012;**188**:3469–77.
52. Miao EA, Mao DP, Yudkovsky N *et al*. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci USA* 2010;**107**:3076–80.
53. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. *Nat Rev Immunol* 2013;**13**:397–411.
54. Fink SL, Bergsbaken T, Cookson BT. Anthrax lethal toxin and Salmonella elicit the common cell death pathway of caspase-1-dependent pyroptosis via distinct mechanisms. *Proc Natl Acad Sci USA* 2008;**105**:4312–7.
55. Stowe I, Lee B, Kayagaki N. Caspase-11: arming the guards against bacterial infection. *Immunol Rev* 2015;**265**:75–84.
56. Kayagaki N, Wong MT, Stowe IB *et al*. Noncanonical inflammasome activation by intracellular LPS independent of TLR4. *Science* 2013;**341**:1246–9.
57. Broz P, Ruby T, Belhocine K *et al*. Caspase-11 increases susceptibility to Salmonella infection in the absence of caspase-1. *Nature* 2012;**490**:288–91.
58. De Jong HK, Koh GC, van Lieshout MH *et al*. Limited role for ASC and NLRP3 during in vivo Salmonella typhimurium infection. *BMC Immunol* 2014;**15**:30.
59. Knodler LA, Crowley SM, Sham HP *et al*. Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens. *Cell Host Microbe* 2014;**16**:249–56.
60. Kayagaki N, Stowe IB, Lee BL *et al*. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 2015;**526**:666–71.
61. Oficjalska K, Raverdeau M, Aviello G *et al*. Protective role for caspase-11 during acute experimental murine colitis. *J Immunol* 2015;**194**:1252–60.
62. Casson CN, Yu J, Reyes VM *et al*. Human caspase-4 mediates noncanonical inflammasome activation against gram-negative bacterial pathogens. *Proc Natl Acad Sci USA* 2015; **112**:6688–93.

63. Kayagaki N, Warming S, Lamkanfi M *et al*. Non-canonical inflammasome activation targets caspase-11. *Nature* 2011; **479**:117–21.
64. Xing L, Zhang Z, Ruan J *et al*. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 2016;**535**:153–8.
65. He W, Wan H, Hu L *et al*. Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. *Cell Res* 2015;**25**:1285–98.
66. Ding J, Wang K, Liu W *et al*. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 2016; **535**:111–6.
67. Poreba M, Strózyk A, Salvesen GS *et al*. Caspase substrates and inhibitors. *Cold Spring Harb Perspect Biol* 2013;**5**:a008680.
68. Rühl S, Broz P. The gasdermin-D pore: executor of pyroptotic cell death. *Oncotarget* 2016;**7**:57481–2.
69. Sborgi L, Rühl S, Mulvihill E *et al*. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. *EMBO J* 2016;**35**:1766–78.
70. Wright JA, Bryant CE. The killer protein Gasdermin D. *Cell Death Differ* 2016;**23**:1897–8.
71. Yang J, Zhao Y, Shi J *et al*. Human NAIP and mouse NAIP1 recognize bacterial type III secretion needle protein for inflammasome activation. *Proc Natl Acad Sci USA* 2013;**110**: 14408–13.
72. Zhao Y, Yang J, Shi J *et al*. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* 2011;**477**:596–600.
73. Case CL, Kohler LJ, Lima JB *et al*. Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to Legionella pneumophila. *Proc Natl Acad Sci U S A* 2013;**110**:1851–6.
74. Rühl S, Broz P. Caspase-11 activates a canonical NLRP3 inflammasome by promoting K(+) efflux. *Eur J Immunol* 2015; **45**:2927–36.
75. Jorgensen I, Zhang Y, Krantz BA *et al*. Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis. *J Exp Med* 2016; **213**:2113–28.
76. Jorgensen I, Lopez JP, Laufer SA *et al*. IL-1ADDIN-18, and eicosanoids promote neutrophil recruitment to pore-induced intracellular traps following pyroptosis. *Eur J Immunol* 2016; **46**:2761–6.
77. Ryu JC, Kim MJ, Kwon Y *et al*. Neutrophil pyroptosis mediates pathology of P. aeruginosa lung infection in the absence of the NADPH oxidase NOX2. *Mucosal Immunol* 2017;**10**:757–74.
78. Sauer JD, Pereyre S, Archer KA *et al*. Listeria monocytogenes engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. *Proc Natl Acad Sci USA* 2011;**108**:12419–24.
79. Masters SL, Lagou V, Jéru I *et al*. Familial autoinflammation with neutrophilic dermatosis reveals a regulatory mechanism of pyrin activation. *Sci Transl Med* 2016;**8**:332ra45.
80. Tanaka S, Mizushina Y, Kato Y *et al*. Functional conservation of gsdma cluster genes specifically duplicated in the mouse genome. *G3 (Bethesda)* 2013;**3**:1843–50.
81. Guo H, Xu S, Liu Y *et al*. Gsdma3 is required for mammary gland development in mice. *Histochem Cell Biol* 2017;**147**: 575–83.
82. Chao KL, Kulakova L, Herzberg O. Gene polymorphism linked to increased asthma and IBD risk alters gasdermin-B structure, a sulfatide and phosphoinositide binding protein. *Proc Natl Acad Sci USA* 2017;**114**:E1128–37.
83. Liu N, Su H, Zhang Y *et al*. Cholecalciterol cholesterol emulsion attenuates experimental autoimmune myocarditis in mice via inhibition of the pyroptosis signaling pathway. *Biochem Biophys Res Commun* 2017;**493**:422–8.
84. Pu Q, Gan C, Li R *et al*. Atg7 deficiency intensifies inflammasome activation and pyroptosis in pseudomonas sepsis. *J Immunol* 2017;**198**:3205–13.
85. Raeisi S, Farokhi E, Taherzadeh M *et al*. Study of deafness associated with DFNB59 gene (pejvakin) mutation in Fars province. *Qom Univ Med Sci J* 2012;**4**:10–5.
86. Borck G, Rainshtein L, Hellman-Aharony S *et al*. High frequency of autosomal-recessive DFNB59 hearing loss in an isolated Arab population in Israel. *Clin Genet* 2012;**82**:271–6.
87. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008;**9**:231–41.
88. Bergsbaken T, Cookson BT. Macrophage activation redirects yersinia-infected host cell death from apoptosis to caspase-1-dependent pyroptosis. *PLoS Pathog* 2007;**3**:e161.
89. Man SM, Kanneganti TD. Converging roles of caspases in inflammasome activation, cell death and innate immunity. *Nat Rev Immunol* 2016;**16**:7–21.
90. Davidovich P, Kearney CJ, Martin SJ. Inflammatory outcomes of apoptosis, necrosis and necroptosis. *Biol Chem* 2014;**395**: 1163–71.
91. Creagh EM. Caspase crosstalk: integration of apoptotic and innate immune signalling pathways. *Trends Immunol* 2014; **35**:631–40.
92. Kaplan GG. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* 2015;**12**:720–7.
93. Chen HM, Liu XW, Sun RJ *et al*. A survey on the developmental intestinal microbiota research in China: the history, funding, and frontiers of gut bacteria. *J Dig Dis* 2015;**16**:421–30.
94. Frolkis AD, Dykeman J, Negrón ME *et al*. Risk of surgery for inflammatory bowel diseases has decreased over time: a systematic review and meta-analysis of population-based studies. *Gastroenterology* 2013;**145**:996–1006.
95. Frolkis AD, Lipton DS, Fiest KM *et al*. Cumulative incidence of second intestinal resection in Crohn's disease: a systematic review and meta-analysis of population-based studies. *Am J Gastroenterol* 2014;**109**:1739–48.
96. Wei H, Liu X, Ouyang C *et al*. Complications following stem cell therapy in inflammatory bowel disease. *Curr Stem Cell Res Ther* 2017;**12**:471–5.
97. Liu G, Lin J, Xie H *et al*. Histomorphological features and prognosis of colitis-associated colorectal cancer in patients with primary sclerosing cholangitis. *Scand J Gastroenterol* 2015;**50**:1389–96.
98. Lei-Leston AC, Murphy AG, Maloy KJ. Epithelial cell inflammasomes in intestinal immunity and inflammation. *Front Immunol* 2017;**8**:1168.
99. Liston A, Masters SL. Homeostasis-altering molecular processes as mechanisms of inflammasome activation. *Nat Rev Immunol* 2017;**17**:208–14.
100. Davis BK, Philipson C, Hontecillas R *et al*. Emerging significance of NLRs in inflammatory bowel disease. *Inflamm Bowel Dis* 2014;**20**:2412–32.
101. Allen IC, TeKippe EM, Woodford RM *et al*. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J Exp Med* 2010;**207**:1045–56.
102. Williams TM, Leeth RA, Rothschild DE *et al*. The NLRP1 inflammasome attenuates colitis and colitis-associated tumorigenesis. *J Immunol* 2015;**194**:3369–80.
103. Williams TM, Leeth RA, Rothschild DE *et al*. Caspase-11 attenuates gastrointestinal inflammation and experimental colitis pathogenesis. *Am J Physiol Gastrointest Liver Physiol* 2015;**308**:G139–50.

104. Zaki MH, Boyd KL, Kastan MB *et al*. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. *Immunity* 2010;**32**:379–91.
105. Fu J, Wei B, Wen T *et al*. Loss of intestinal core 1-derived O-glycans causes spontaneous colitis in mice. *J Clin Invest* 2011;**121**:1657–66.
106. Zaki MH, Vogel P, Body-Malapel M *et al*. IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. *J Immunol* 2010;**185**:4912–20.
107. Dupaul-Chicoine J, Yeretssian G, Doiron K *et al*. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 2010;**32**:367–78.
108. Demon D, Kuchmiy A, Fossoul A *et al*. Caspase-11 is expressed in the colonic mucosa and protects against dextran sodium sulfate-induced colitis. *Mucosal Immunol* 2014;**7**:1480–91.
109. Flood B, Oficjalska K, Laukens D *et al*. Altered expression of caspases-4 and -5 during inflammatory bowel disease and colorectal cancer: diagnostic and therapeutic potential. *Clin Exp Immunol* 2015;**181**:39–50.
110. Motta JP, Martin L, Vergnolle N. Proteases/Antiproteases in Inflammatory Bowel Diseases. In: Kamihira M, Katakura Y, Ito A (eds). *Animal Cell Technology: Basic & Applied Aspects*. Basel: Springer, 2011, 173–215.
111. Ishimoto Y, Satsu H, Mochizuki T *et al*. In vitro analysis of the interaction between human intestinal epithelial cells and macrophage-like cells. *Springer Netherlands* 2010;**16**:231–6.
112. Marim FM, Franco MMC, Gomes MTR *et al*. The role of NLRP3 and AIM2 in inflammasome activation during Brucella abortus infection. *Semin Immunopathol* 2017;**39**:215–23.
113. Sharma D, Kanneganti TD. The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation. *J Cell Biol* 2016;**213**:617–29.
114. Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016;**16**:407–20.
115. Lissner D, Schumann M, Batra A *et al*. Monocyte and M1 macrophage-induced barrier defect contributes to chronic intestinal inflammation in IBD. *Inflamm Bowel Dis* 2015;**211**:297–305.
116. Gurung P, Li B, Subbarao Malireddi RK *et al*. Chronic TLR stimulation controls NLRP3 inflammasome activation through IL-10 mediated regulation of NLRP3 expression and caspase-8 activation. *Sci Rep* 2015;**5**:14488.
117. Thinwa J, Segovia JA, Bose S *et al*. Integrin-mediated first signal for inflammasome activation in intestinal epithelial cells. *J Immunol* 2014;**193**:1373–82.
118. Bauer C, Duewell P, Mayer C *et al*. Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* 2010;**59**:1192–9.
119. Ng J, Hirota SA, Gross O *et al*. Clostridium difficile toxin-induced inflammation and intestinal injury are mediated by the inflammasome. *Gastroenterology* 2010;**139**:542–52.
120. Lee HM, Yuk JM, Kim KH *et al*. Mycobacterium abscessus activates the NLRP3 inflammasome via Dectin-1-Syk and p62/SQSTM1. *Immunol Cell Biol* 2012;**90**:601–10.
121. Kim DJ, Park JH, Franchi L *et al*. The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1β production in Helicobacter pylori infected dendritic cells. *Eur J Immunol* 2013;**43**:2650–8.
122. Hirota SA, Ng J, Lueng A *et al*. NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. *Inflamm Bowel Dis* 2011;**17**:1359–72.
123. Bauer C, Duewell P, Lehr HA *et al*. Protective and aggravating effects of Nlrp3 inflammasome activation in IBD models: influence of genetic and environmental factors. *Dig Dis* 2012;**30**:82–90.
124. Lin Z, Hegarty JP, John G *et al*. NOD2 mutations affect muramyl dipeptide stimulation of human B lymphocytes and interact with other IBD-associated genes. *Dig Dis Sci* 2013;**58**:2599–607.
125. Seo S-U, Kamada N, Muñoz-Planillo R *et al*. Distinct commensals induce interleukin-1β via NLRP3 inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury. *Immunity* 2015;**42**:744–55.
126. Cunha LD, Zamboni DS. Subversion of inflammasome activation and pyroptosis by pathogenic bacteria. *Front Cell Infect Microbiol* 2013;**3**:76.
127. Sellin M, Müller A, Felmy B *et al*. Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict salmonella replication in the intestinal mucosa. *Cell Host Microbe* 2014;**16**:237–48.
128. Duprez L, Wirawan E, Vanden Berghe T *et al*. Major cell death pathways at a glance. *Microbes Infect* 2009;**11**:1050–62.
129. He S, Wang L, Miao L *et al*. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* 2009;**137**:1100–11.
130. Cho YS, Challa S, Moquin D *et al*. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* 2009;**137**:1112–23.
131. Rogler G. Update in inflammatory bowel disease pathogenesis. *Curr Opin Gastroenterol* 2004;**20**:311–7.
132. Aguilera M, Darby T, Melgar S. The complex role of inflammasomes in the pathogenesis of inflammatory bowel diseases—lessons learned from experimental models. *Cytokine Growth Factor Rev* 2014;**25**:715–30.
133. Xiong Y, Lou Y, Su H *et al*. Cholecalciterol cholesterol emulsion ameliorates experimental colitis via down-regulating the pyroptosis signaling pathway. *Exp Mol Pathol* 2016;**100**:386–92.