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Pyroptosis: host cell death and inflammation

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Abstract

Eukaryotic cells can initiate several distinct programmes of self-destruction, and the nature of the cell death process (non-inflammatory or proinflammatory) instructs responses of neighbouring cells, which in turn dictates important systemic physiological outcomes. Pyroptosis, or caspase 1-dependent cell death, is inherently inflammatory, is triggered by various pathological stimuli, such as stroke, heart attack or cancer, and is crucial for controlling microbial infections. Pathogens have evolved mechanisms to inhibit pyroptosis, enhancing their ability to persist and cause disease. Ultimately, there is a competition between host and pathogen to regulate pyroptosis, and the outcome dictates life or death of the host.

Cells can die through distinct biochemical pathways that produce different morphological and physiological outcomes. Apoptosis is perhaps the most widely recognized programme of cell death, and is mechanistically defined by the requirement for particular cysteine-dependent aspartate-specific proteases, or caspases, which produce an orchestrated disassembly of the cell[1]. Apoptotic caspases cleave cellular substrates, resulting in the characteristic features of apoptosis, which include cytoplasmic and nuclear condensation, DNA cleavage and maintenance of an intact plasma membrane. The contents of apoptotic cells are packaged into membrane-enclosed apoptotic bodies, which are targeted for phagocytosis and removal *in vivo*, resulting in an absence of inflammation[2] (BOX 1).

Although apoptosis was the first well-recognized programme of eukaryotic cell death, 'programmed cell death' is broadly applied to several endogenous genetically defined pathways in which the cell plays an active part in its own destruction[3]. Other cell death programmes include autophagy, oncosis and caspase 1-dependent programmed cell death (also known as pyroptosis). Pyroptosis is a more recently identified pathway of host cell death that is stimulated by a range of microbial infections (for example, *Salmonella*, *Francisella* and *Legionella*) and non-infectious stimuli, including host factors produced during myocardial infarction[4]. Caspase 1 was first recognized as a protease that processes the inactive precursors of interleukin 1β (IL-1β) and IL-18 into mature inflammatory cytokines, and was initially called interleukin IL-1β-converting enzyme[5]. However, caspase 1 activation can result not only in the production of activated inflammatory cytokines, but also rapid cell death characterized by plasma-membrane rupture and release of proinflammatory intracellular contents[6,7]. Caspase 1-dependent cell death is a programmed process of cellular self-destruction mediated by caspases, and therefore was not initially distinguished from apoptosis[8–11]. However, the mechanism, characteristics and outcome of caspase 1-dependent cell death are distinct from apoptosis[6,7,12]. Thus, the term pyroptosis

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(from the Greek ‘pyro’, relating to fire or fever, and ‘ptosis’, meaning a falling (BOX 1)), is used to described the inherently inflammatory process of caspase 1-dependent programmed cell death[13].

Mechanism and features of pyroptosis

Pyroptosis is morphologically and mechanistically distinct from other forms of cell death. Caspase 1 dependence is a defining feature of pyroptosis, and caspase 1 is the enzyme that mediates this process of cell death (FIG. 1). Caspase 1 is not involved in apoptosis, and caspase 1-deficient mice have no defects in apoptosis and develop normally[14,15]. The apoptotic caspases, including caspase 3, caspase 6 and caspase 8, are not involved in pyroptosis[6,10,12,16–20], and substrates of apoptotic caspases, including poly (ADP-ribose) polymerase and inhibitor of caspase-activated DNase (ICAD), do not undergo proteolysis during pyroptosis[6,7,9,12]. Furthermore, loss of mitochondrial integrity and release of cytochrome *c*, which can activate apoptotic caspases, do not occur during pyroptosis[16,19].

Pyroptosis features rapid plasma-membrane rupture and release of proinflammatory intracellular contents. This is in marked contrast to the packaging of cellular contents and non-inflammatory phagocytic uptake of membrane-bound apoptotic bodies that characterizes apoptosis[2]. Cell lysis during pyroptosis results from caspase 1-mediated processes[8,9,12,17,18,20–24]. *Salmonella* infection or treatment with lethal toxins from Bacillus anthracis produces plasma-membrane pores with a functional diameter of 1.1–2.4 nm[7,20], and pore formation is a host cell-mediated process that is dependent on caspase 1 activity[7,12,20]. Caspase 1-dependent plasma-membrane pores dissipate cellular ionic gradients, producing a net increased osmotic pressure, water influx, cell swelling and, eventually, osmotic lysis and release of inflammatory intracellular contents[7]. Indeed, cells dying by pyroptosis undergo a measurable size increase[7,18] (FIG. 1). In support of this mechanism, the cytoprotective agent glycine non-specifically blocks ion fluxes in damaged eukaryotic cells and thereby prevents swelling and lysis during pyroptosis[6,7,21,25,26].

Cleavage of chromosomal DNA is a fatal event that is often assumed to indicate apoptotic cell death[3]; however, DNA damage also occurs during pyroptosis[6,12,24,27,28]. During apoptosis, caspase-mediated proteolysis of ICAD releases caspase-activated DNase (CAD). CAD cleaves DNA between nucleosomes, resulting in characteristic oligonucleosomal DNA fragments of approximately 180 bp[7]. Although purified caspase 1 can cleave ICAD *in vitro*[11], ICAD degradation does not occur during pyroptosis[7,12]. DNA cleavage during pyroptosis instead results from the activity of an unidentified caspase 1-activated nuclease that does not produce the oligonucleosomal DNA fragmentation pattern that is characteristic of apoptosis[7,12,29]. DNA cleavage is accompanied by marked nuclear condensation, but unlike apoptosis, nuclear integrity is maintained[12,23] (FIG. 1). DNA cleavage and cell lysis are both caspase 1-dependent features of pyroptosis, but cell lysis does not require DNA cleavage[7].

Destruction of the actin cytoskeleton has also been observed in cells undergoing pyroptosis, but the mechanism and importance of this destruction remains unclear[12,26]. Caspase 1-dependent degradation of cellular inhibitor of apoptosis protein (cIAP) also accompanies during pyroptosis, although the exact mechanism that underlies cIAP degradation is also unknown[30]. Caspase 1 cleaves and inactivates metabolic enzymes during pyroptosis31, and identification of additional proteolytic targets of caspase 1 could yield insight into the mechanism of pyroptosis and novel features of this form of cell death.

Box 1 | Apoptosis is a programmed process that results in non-inflammatory cell death

Hippocrates was the first to use the term apoptosis in the medical literature (approximately 460–370 BCE)[121]. After years of exhaustive microscopic evaluation, apoptosis was reintroduced by Kerr *et al.* in 1972 to describe an active, programmed process that leads to cell death in both healthy and diseased tissues122. Its morphological characteristics included condensation of both the cytoplasm and the nucleus, and the generation of cell fragments called apoptotic bodies, which were phagocytosed by intact cells and subsequently destroyed. Little tissue disruption and a marked lack of inflammation suggested the process was a "general mechanism of controlled cell deletion, which is complementary to mitosis in the regulation of animal cell populations." (REF. 122) Cell death caused by apoptosis was previously referred to as shrinkage necrosis. By contrast, coagulative necrosis was "invariably caused by noxious stimuli" and resulted from "irreversible disturbance of cellular homeostatic mechanisms." (REF. 122) These original descriptions are consistent with recent recommendations for using nomenclature that defines cell death, or necrosis, as the end product of processes such as apoptosis3,123. The term apoptosis, which in Greek is used to describe the 'falling off' of leaves from a tree, was suggested to indicate the controlled loss of individual cells from the population. Pronunciation provides a clear indication of its Greek roots: "we propose that the stress should be on the penultimate syllable, the second half of the word being pronounced like "ptosis" (with the "p" silent), which comes from the same root "to fall" and is already used to describe drooping of the upper eyelid." (REF. 122) The ultrastructural features described in this landmark paper are still considered to be hallmarks of apoptosis, and subsequent research has identified the important role of a subset of caspases in mediating the morphological changes observed in this and other early studies1.

TLRs and NLRs

The host can use a range of mechanisms to sense intracellular and extracellular 'danger' signals generated by invading pathogenic microorganisms or by the host in response to tissue injury[32]. Toll-like receptors (TLRs) initiate a signalling cascade that leads to cellular activation and production of inflammatory cytokines, such as tumour necrosis factor (TNF), IL-6, IL-8 and type I interferons (IFNs), in response to extracellular signals[33] (FIG. 2). Nod-like receptors (NLRs) function in the recognition of danger signals introduced into the host cell cytosol[34]. The NLRs nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2 trigger a signalling cascade following ligand recognition that, similarly to the cascade initiated by TLRs, results in inflammatory cytokine production[34] (FIG. 2). Another subset of NLRs trigger activation of the cysteine protease caspase 1 (REF. 35), which leads to caspase 1-dependent pyroptosis and processing and release of the inflammatory cytokines IL-18 and IL-1β3 (FIG. 2). TLRs and caspase 1-activating NLRs often act in concert with TLR stimulation, resulting in enhanced susceptibility to NLR-mediated caspase 1 activation in response to ATP treatment[36– 38] and *Yersinia* infection[12]. TLRs and NOD1 and NOD2 also stimulate the production and intracellular accumulation of pro-IL-1β[33,34]. Thus, TLRs and NOD1 and NOD2 prime cells to undergo caspase 1 activation and produce maximal IL-1β in response to subsequent cytosolic recognition of host- or pathogen-derived danger signals.

Caspase-1-activating NLRs

NLR recognition of bacterial, viral and host molecules, as well as toxic foreign products, can lead to the activation of caspase 1. The NLR protein NLRP3 (NACHT, LRR and PYD domains-containing protein 3; also known as NALP3) responds to multiple stimuli, including pore-forming toxins[38–40], extracellular ATP in the presence of various pathogen-associated molecules38,41,[42], uric acid crystals[43], virus-associated DNA[44], RNA[45],

asbestos[46] and ultraviolet B irradiation[47]. The mechanism by which NLRP3 detects this divergent group of signals is unknown. Cellular potassium efflux is a common response to many of these stimuli, and preventing potassium efflux blocks caspase 1 activation48–50. However, potassium efflux alone does not seem to be sufficient to trigger activation of caspase 1 (REFS 48,51), and preventing potassium efflux also blocks caspase 1 activation that is mediated by another NLR, NLRP1b (also known as NALP1b)[20,52,53]. This indicates that potassium efflux may not directly signal for NLRP3-dependent caspase 1 activation, but rather creates an environment that is favourable for ligand detection and/or caspase 1 activation49,[52,54]. It is possible that host cells respond to all of these stimuli by generating one or more secondary factors that bind NLRP3, and further experiments are needed to determine how NLRP3 directly recognizes or participates in the response to such a broad range of molecules.

The NLR protein NLRC4 (NLR family CARD domain-containing protein 4; also known as IPAF) mediates the recognition of diverse bacterial pathogens, which during infection reside extracellularly (for example, *Pseudomonas*) or intracellularly (for example, *Salmonella*, *Legionella*, *Listeria* and *Shigella*), and share similar requirements for the activation of caspase 1. These pathogens deliver virulence determinants into host cells through translocation systems that form conduits between the bacteria and host cell cytosol. The same conduits, key to the pathogenesis of infection, also betray the presence of pathogens by introducing flagellin into the host cell, where its recognition is facilitated by NLRC4 (REFS 23,55–59). During infection with cytosolic pathogens, such as *Listeria*, secreted flagellin has direct access to the cytosol, and a translocation system is not required[60]. Expression of flagellin in the macrophage cytosol stimulates NLRC4-dependent pyroptosis[61], suggesting that NLRC4 directly recognizes flagellin; however, such an interaction has not been demonstrated. Interestingly, NLRC4-dependent caspase 1 activation has been reported during infection with *Pseudomonas* and *Shigella* mutants that do not produce flagellin[62,63]. These studies suggest that NLRC4, like NLRP3, can respond to additional bacterial components that remain unidentified.

The NLR NLRP1b recognizes cytosolic delivery of *B. anthracis* lethal toxin, a metalloprotease that can cleave host mitogen-activated protein kinases (MAPKs). NLRP1b-mediated caspase 1 activation is not due to structural recognition of the toxin itself, as lethal toxin that contains a point mutation in the catalytic site, but retains its native structure, fails to activate caspase 1 (REFS 20,64). Proteolytic activity of lethal toxin is required for caspase 1 activation, but MAPK cleavage alone is not sufficient, suggesting that as-yet-unidentified lethal toxin substrates are involved20. Proteasome activity is also required for caspase 1 activation in response to lethal toxin treatment20,[30,53], suggesting that a lethal toxin-mediated alteration in proteasome function allows caspase 1 activation[30].

Several NLR proteins, in addition to those described above, have been implicated in caspase 1 activation[35]. The NLR neuronal apoptosis inhibitory protein 5 (NAIP5) is required for caspase 1 activation during infection with *Legionella*, but does not seem to be necessary for all bacteria that activate caspase 1 through NLRC4 (REF. 61), and the exact role of NAIP5 in pyroptosis is unknown. *Francisella* requires ASC (apoptosis-associated speck-like protein containing a CARD), but not NLRC4 or NLRP3, to stimulate caspase 1 activation[24,38], which implicates another NLR in the recognition of this pathogen.

The inflammasome

NLRs recognize their cognate host- or microorganism-derived danger signals and trigger formation of a multiprotein complex called the inflammasome, which contains caspase 1 (REFS 35,65). NLRs that have encountered their signal undergo nucleotide-dependent

oligomerization using their nucleotide-binding domain66. Some NLRs, including NLRP3, bind to the adapter protein ASC, which contains a caspase activation and recruitment domain (CARD) and interacts with caspase 1 (REF. 35) (FIG. 3a). Other NLRs, such as NLRC4, contain a CARD and can directly interact with caspase 1 when overexpressed[67] (FIG. 3a). The association of caspase 1 within this complex allows its processing and activation[35].

It has been proposed that a single NLR mediates caspase 1 activation in response to a given stimulus, and these complexes can be observed *in vitro*[65,66]. However, other data suggest that interactions between multiple NLRs might contribute to inflammasome formation. For example, NAIP5 can affect the ability of *Legionella* to stimulate NLRC4-dependent activation of caspase 1 (REF. 61). NAIP5 can bind NLRC4 and contains a pathogen-sensing leucine-rich-repeat (LRR) domain57, but its exact role in inflammasome formation is unknown. NAIP5 does not seem to play a part in all NLRC4-containing inflammasomes, as NAIP5 is not required for caspase 1 activation by *Salmonella*[61]. Similarly, both NLRP3 and NLRC4 have a role in caspase 1 activation in response to *Listeria* infection[60], pore-forming toxins[39] and ultraviolet B irradiation[47]. These data suggest that multiple sensors are present in the same complex and function cooperatively to activate caspase 1. In addition, microbial infection could lead to cell damage and release of host danger signals, such as uric acid and ATP, that stimulate the activation of caspase 1. However, the release of these host ligands by dying cells has not been shown *in vivo*. Thus, host cells encounter a barrage of caspase 1-activating ligands and are endowed with a diverse sensor array to trigger the common downstream response of pyroptosis efficiently[20].

Inflammasomes were observed microscopically during *Salmonella* infection and treatment with *B. anthracis* lethal toxin, and active caspase 1 was found to be located within a single inflammasome complex as well as diffusely distributed throughout the cytoplasm[20] (FIG. 3b). The adapter protein ASC can self-associate and form similarly sized complexes in the absence of an NLR[54], but the extent to which the self-association of ASC contributes to the formation of NLR-containing inflammasomes is unknown (FIG. 3b). However, the fact that *Salmonella*-mediated activation of caspase 1 is reduced in ASC-deficient macrophages[68] suggests that ASC facilitates caspase 1 activation even though it is not absolutely required for the binding of NLRC4 to caspase 1 (FIG. 3a). These data are consistent with the formation of a single, large inflammasome, or aggregation of multiple complexes that contain one or more NLRs, rather than many smaller complexes within a cell. The localization of a large percentage of active caspase 1 within a single complex could limit access to some caspase 1 substrates and allow recruitment of others by a mechanism that is analogous to recruitment of substrates to the proteasome. By this model, regulatory proteins could recruit substrates, control access to the proteolytic regions of the complex and alter the enzymatic function of the complex to regulate substrate cleavage[69]. Similarly, the catalytic activity of caspase 9 is enhanced when it is bound to the apoptosome, a multiprotein complex that is involved in caspase 9 activation[70]. Defining the components of native inflammasomes will provide insight into how this complex functions in its regulation of caspase 1 activity.

Inflammasome components can also interact with proteins that activate alternative cellular processes or forms of cell death. Autophagy has been observed during infection of macrophages with *Legionella*[71,72] and *Francisella*[73], which can also induce caspase 1 under other *in vitro* conditions[23,24]. Failure to induce robust caspase 1 activation owing to suboptimal ligand production by the pathogen or host mutations does not result in pyroptosis, but instead may allow inflammasome components to interact with other cell death machinery and stimulate alternative cell death pathways[23,72]. ASC- and caspase 1-deficient macrophages fail to activate caspase 1 in response to multiple stimuli, but are not

always protected from cell lysis, suggesting that the absence of caspase 1-dependent pyroptosis allows other cell death processes to predominate, including pyronecrosis and autophagy[62,63,74,124]. Infection with *Shigella* or *Salmonella* triggers caspase 1 activation in wild-type macrophages, but in the absence of caspase 1, infected macrophages display features of autophagy[63,75]. The induction of autophagy by *Shigella* requires the NLR protein NLRC4, implicating NLR proteins in stimulation of both pyroptosis and autophagy[63].

Caspase 1-dependent processes

Several caspase 1-dependent processes do not directly contribute to cellular demise, but accompany the cell death process and contribute to the inflammatory nature of pyroptosis. In addition, some events that are caspase 1-dependent can occur in the absence of cell death. Caspase 1 activation can result in a combination of the following processes, which are dictated by cell type as well as the nature and magnitude of the stimulus received.

IL-1β and IL-18 processing and secretion

The inflammatory cytokines IL-1β and IL-18 undergo caspase 1-dependent activation and secretion during pyroptosis. IL-1β is a potent endogenous pyrogen that stimulates fever, leukocyte tissue migration and expression of diverse cytokines and chemokines[76]. IL-18 induces IFNγ production and is important for the activation of T cells, macrophages and other cell types[77]. Both IL-1β and IL-18 play crucial parts in the pathogenesis of a range of inflammatory and autoimmune diseases[76,77]. Although neither cytokine is required for the process of cell death[37,78], their production contributes to the inflammatory response elicited by cells undergoing pyroptosis. IL-1β and IL-18 lack secretion signals and their mechanism of release has not been definitively determined. Formation of caspase 1-dependent pores in the plasma membrane is temporally correlated with cytokine release in macrophages[7], suggesting that cytokine secretion occurs through these pores (FIG. 1). Interestingly, lysis is not required for the release of activated IL-1β and IL-18, because pharmacological inhibition of lysis does not prevent caspase 1-dependent pore formation or cytokine secretion[7]. Thus, cytokine secretion and cell lysis are both downstream consequences of caspase 1-dependent pore formation (FIG. 1).

Additional mechanisms of IL-1β and IL-18 release have also been described that occur in the absence of cell lysis. Monocytes package active caspase 1 and cytokine substrates into lysosomes[79,80], and secretion of processed cytokines occurs through lysosome fusion with the cell surface[80] (FIG. 1). Although this is an elegant mechanism for cytokine secretion in the absence of pyroptosis, recent evidence suggests this may be limited to monocytes[81]. Release of cytokine-containing vesicles has also been observed in a range of cell types, including dendritic cells, microglial cells and macrophages, during caspase 1 activation in response to treatment with ATP[82–85]. Two mechanisms have been proposed for vesicle release: fusion of multivesicular bodies with the cell surface[82] and direct budding of microvesicles from the plasma membrane[83–85] (FIG. 1). Vesicle release has so far only been observed in response to ATP stimulation, and surface microvesicle shedding results in a significant reduction in cell size owing to loss of the plasma membrane[83,85]. By contrast, in *Salmonella*- and *Burkholderia*-infected macrophages, cells increase in size as processed cytokines are released[7,18], suggesting that alternative mechanisms also mediate secretion of IL-1β and IL-18.

Additional inflammatory cytokines

Caspase 1 activation is also required for maximal production of inflammatory cytokines other than IL-1β and IL-18. Active caspase 1 has been shown to bind to and facilitate secretion of IL-1α by an unknown mechanism[5,86]. A modest but significant reduction in

TNF and IL-6 secretion by caspase 1-deficient macrophages in response to lipopolysaccharide stimulation has also been reported[14,15,87]. This is due to caspase 1-mediated cleavage of the TLR adapter protein TIRAP (Toll/interleukin-1 receptor domain-containing adapter protein; also known as MAL). Caspase 1-processed TIRAP signals more efficiently, resulting in enhanced TNF and IL-6 production and macrophage activation in response to TLR2 and TLR4 ligands[87]. Therefore, in addition to regulating the production of IL-1β and IL-18, caspase 1 activation can also have a role in fine-tuning cytokine responses to microbial stimuli.

Inhibiting growth of intracellular bacteria

Caspase 1 activation helps to restrict the growth of intracellular pathogens. In macrophages that fail to trigger robust caspase 1 activation in response to *Legionella* infection, the bacteria replicate within an endoplasmic reticulum-derived compartment that resembles an immature autophagosome[71]. Infection of macrophages that more readily activate caspase 1 results in the rapid caspase 1-dependent delivery of *Legionella* to lysosomes and degradation of the bacteria[23,88]. Caspase 1 activity also enhances the killing of mycobacteria by stimulating trafficking of the bacteria to lysosomal compartments[89]. However, caspase 1 is not required for the degradation of all bacteria[88]. *Legionella*, mycobacteria and other pathogens produce virulence factors that modulate the trafficking of intracellular compartments, and further experiments are required to determine how caspase 1 allows macrophages to overcome these bacterial factors and contributes to the control of pathogen replication *in vivo*.

Cell repair and survival

Caspase 1 activation fails to trigger pyroptosis in all cell types, and somewhat surprisingly, epithelial cells use caspase 1 activation to prevent cell death[39]. Caspase 1 activation stimulates lipid production and membrane repair in response to the pore-forming toxins aerolysin and α-toxin, and indeed inhibition of caspase 1 activity actually enhances cell lysis[39]. This suggests that under certain conditions activation of caspase 1 could represent a cellular survival mechanism.

The function of caspase 1 is analogous to the activities of other apoptotic caspases (caspases 3 and 8) in modulating the fate of certain cell types[90]. Low levels of apoptotic caspase activation prevent autophagic cell death, regulate the proliferation and differentiation of B and T cells, and control the maturation of dendritic cells90. Higher levels of activation of the same apoptotic caspases result in the non-inflammatory elimination of these cells90. Similarly, the magnitude of caspase 1 activation modulates the response to microbial stimuli and host factors that warrant an inflammatory response. Low levels of active caspase 1 stimulate cell survival responses, control intracellular bacterial growth and mediate inflammatory cytokine production. When caspase 1 activation passes a critical threshold level, cells undergo pyroptosis and release inflammatory intracellular contents.

We propose that the level of caspase 1 activation tailors the host response to inflammatory stimuli. In addition, the fate of cells with active caspase 1 could be controlled independently of active enzyme levels by the subcellular localization of caspase 1. Restriction of active caspase 1 to lysosomes by monocytes[79,80] could sequester certain substrates to one compartment for cleavage and release, while keeping cellular substrates that mediate cell death in another. *In vivo*, minimizing pyroptosis and intravascular lysis of circulating monocytes would probably be crucial to avoid an unfocused and potentially lethal systemic inflammatory response. The function of active caspase 1 could also be regulated by its localization within the cytosol. The confinement of active caspase 1 to a single focus within the cell cytosol has been observed[20,54] (FIG. 3b), and this restricted localization could limit

the access of the active enzyme to certain cellular substrates, as previously discussed. The molecular decision to undergo pyroptosis could be modulated by the presence of death effector proteins within a given cell type. Cells are not uniformly susceptible to this process: several stimuli that trigger pyroptosis in macrophages and dendritic cells fail to do so in epithelial cells[39,91].

Caspase 1 in host response and disease pathology

Pyroptosis protects against infection and induces pathological inflammation. Although caspase 1 activity and pyroptosis can have a role as a protective host response to infectious diseases, exuberant or inappropriate caspase 1 activation and pyroptosis can be detrimental (FIG. 4). Mutations in NLR proteins can lead to inappropriate caspase 1 activation, which is associated with hereditary autoinflammatory syndromes[92]. Furthermore, caspase 1 is involved in the pathogenesis of several diseases, including myocardial infarction[4], cerebral ischaemia[93], inflammatory bowel disease[94], neurodegenerative diseases95 and endotoxic shock14, each of which are characterized by inflammation and cell death. Caspase 1 deficiency, or pharmacological inhibition, provides protection against the inflammation, cell death and organ dysfunction that are associated with these diseases, making caspase 1 an attractive therapeutic target. The protection afforded by caspase 1 deficiency against sepsis and renal failure is not mimicked by neutralization of the cytokine targets, IL-1β and IL-18 (REFS 96–98), suggesting that caspase 1 has an additional role in disease apart from cytokine processing.

Caspase 1 activation helps to clear pathogens, such as *Salmonella*[99],100, *Shigella*[101], *Legionella*[23,57], *Francisella*[24], Anaplasma phagocytophilum[102] and *Listeria*[103], during infection *in vivo* in response to innate immune recognition of microorganism-associated patterns. This phenotype cannot be attributed solely to IL-18 and IL-1β production. Mice that are deficient in caspase 1 are more susceptible to *Francisella* than mice that lack both IL-1β and IL-18, indicating that cell death itself, or other caspase 1-dependent processes, contributes to the control of infection[104].

Caspase 1 activation also influences the development of adaptive immune responses. In conjunction with IL-12, IL-18 plays a major part in stimulating the differentiation of T helper 1 (T_H1)-type $CD4^+$ T cells and enhancing their IFNγ production[5,77]. Caspase 1-deficient mice infected with Candida albicans, *Listeria* or *A. phagocytophilum* have an impaired T_H1 response compared with wild-type mice[102,103,105]. $CD4^+$ T cells generated in caspase 1-deficient mice during infection shift towards a T_H2 phenotype[102,103,105], resulting in impaired resistance to secondary infection by pathogens for which T_H1-type responses are required for immunity[105]. The ability of caspase 1 activation to enhance the development of adaptive immune responses is supported by the finding that the non-microbial activators of caspase 1 can act as adjuvants. Uric acid released from necrotic cells enhances cross-presentation and generation of $CD8^+$ T cells that are specific for exogenous antigens[106]. Aluminium-containing adjuvants also stimulate caspase 1 activation[107] and lead to T_H2 $CD4^+$ T cells and robust humoral immune responses[108]. Mice that cannot activate caspase 1 in response to aluminium-containing adjuvants fail to recruit inflammatory cells[109] and cannot stimulate T_H2 $CD4^+$ T-cell responses[109,110]. However, the role of caspase 1 in the regulation of antibody production remains controversial[109–112]. The contributions of pyroptosis to host resistance are therefore multifaceted. Early in infection, caspase 1-mediated processes, including, but not limited to, IL-1β and IL-18 production, lead to activation and recruitment of immune cells and innate control of infection. During persistent infection, continued caspase 1-dependent inflammation promotes the development of appropriate adaptive immune responses that lead to the resolution of infection (FIG. 4).

Microbial regulation of caspase 1 activation

Active caspase 1 allows the host to control various microbial infections, so it is not surprising that pathogens have evolved mechanisms to limit the activation of caspase 1 in response to infection. Innate recognition is often limited to microbial patterns that are required for pathogen survival, such as peptidoglycan, lipopolysaccharide, and nucleic acids[33,34]. Flagellin, which is recognized by NLRC4, is not required for the survival or virulence of *Salmonella* or *Legionella in vivo*[23,113]. *Legionella* and *Salmonella* use translocation systems to modulate host cell function, but must also avoid introducing flagellin into the cytosol through these translocation systems and stimulating pyroptosis. Both organisms downregulate flagellin production during intracellular growth[114,115], which could provide a strategy to avoid pyroptosis, thereby limiting inflammation and allowing continued intracellular replication of the bacteria.

There are multiple examples of pathogens that induce an alternative form of cell death, effectively eliminating cells that would otherwise undergo pyroptosis and stimulate pathogen clearance. Apoptosis kills macrophages by a process that results in the production of anti-inflammatory factors and maintains membrane integrity, thereby preventing release of inflammatory intracellular contents[2]. The activation of apoptotic caspase 3 also results in cleavage of the caspase 1 substrate IL-18 at an alternate site, rendering it non-inflammatory[5]. *Yersinia* can trigger apoptosis in naive macrophages and dendritic cells, which effectively prevents inflammatory pyroptosis[12] (FIG. 5). *Pseudomonas* strains that produce the type III secretion system-secreted protein ExoU induce caspase 1-independent necrosis, resulting in lysis but preventing the cleavage and release of IL-1β and IL-18 (REF. 62). However, 80% of clinical isolates are ExoU negative62, and instead trigger pyroptosis58,59,[62] (FIG. 5). *Pseudomonas* strains that express ExoU are more virulent[62], supporting the hypothesis that neutralizing macrophages before they have the opportunity to activate caspase 1 benefits the bacteria during infection.

Pathogens also produce factors that can directly inhibit the activation of caspase 1. Poxviruses are DNA viruses that replicate in the cytoplasm and would therefore be readily detected by NLRP3. The poxvirus protein M13L-PYD binds ASC through its pyrin domain (FIG. 3a), thereby disrupting inflammasome formation and preventing binding to and activation of caspase 1. Deletion of this viral protein results in increased caspase 1 activity and impaired replication in host cells *in vitro* and during infection *in vivo*[116]. The influenza virus protein NS1 has also been shown to limit caspase 1 activation and cell death by an unknown mechanism[117], which indicates that inhibition of caspase 1 activation could be a common strategy for successful viral pathogens. *Yersinia* translocates type III secretion proteins that counteract the caspase 1 activating potential of the type III secretion system itself. *Yersinia* strains that lack all the type III secretion system-translocated proteins have an increased ability to activate caspase 1 (REFS 12,91,118). Analysis of individual effectors suggests that YopE has an important role in the inhibition of caspase 1 activation, probably owing to the ability of YopE to modulate host Rho GTPase function118. *Francisella* mutants that trigger induction of pyroptosis more quickly than the wild type have been identified, suggesting that *Francisella* also possesses a mechanism for inhibiting caspase 1 (REF. 119), and Mycobacterium tuberculosis produces a zinc metalloprotease that prevents activation of caspase 1 through an unknown mechanism[89]. Finally, mutants of *Francisella* and *Mycobacterium* that cannot control caspase 1 activation are attenuated *in vivo*, which is consistent with the idea that increased levels of active caspase 1 and pyroptosis limit bacterial replication[89,119] (FIG. 5).

Host cell activation redirects cell death

Caspase 1 activation clearly functions as a host defence mechanism in a wide range of microbial infections. Although localized inflammation during infection could enhance tissue disruption and pathogen dissemination, as infection progresses, caspase 1 activation limits pathogen replication, enhances innate and adaptive immune responses, and improves host survival (FIG. 4). Pathogens require mechanisms for preventing or controlling the potent inflammatory outcome of pyroptosis to persist and cause disease. Likewise, the host has evolved mechanisms to counteract pathogen-mediated regulation of caspase 1 activity and successfully control infection. Activation of macrophages counteracts *Yersinia*-mediated inhibition of pyroptosis[12] and enhances susceptibility to *Francisella*-induced pyroptosis[120]. Host recognition of microbial infection may lead to upregulation of NLRs or other unknown accessory proteins that are involved in caspase 1 activation and prime macrophages to undergo pyroptosis. This enhanced sensitivity to pyroptosis allows a shift from the non- or anti-inflammatory modes of cell death triggered by *Yersinia* and *Francisella* in naive macrophages (apoptosis and autophagy, respectively) to inflammatory pyroptosis (FIG. 5). The transition from autophagy to pyroptosis is also observed during *Legionella* infection, perhaps owing to increased production of flagellin by the bacteria[72]. The ability of macrophage activation to enhance pyroptosis in response to *Legionella* infection remains unexplored. Activation could sensitize *Legionella*-infected cells to undergo pyroptosis in response to lower amounts of flagellin. Together, these data clearly indicate that a host-mediated redirection of cell death to pyroptosis can benefit the host by increasing inflammation and facilitating the resolution of infection.

Concluding remarks

A wide range of host and microbial factors stimulate caspase 1 activation, and this leads to an array of caspase 1-dependent processes that include cell death, modulation of inflammatory cytokine production and restriction of pathogen replication. Together, these caspase 1-dependent processes benefit the host *in vivo* by contributing to the control of microbial infection. Pathogens use virulence factors to limit caspase 1 activation, but the host has mechanisms for priming cells to activate caspase 1 in the presence of this inhibition. Ultimately, there is competition between host and pathogen to regulate caspase 1 activation, and the outcome dictates life or death of the host.

Host and microbial factors that trigger caspase 1 activation, and the host NLR proteins that detect these molecules, have been the focus of recent research. We are only beginning to understand the molecular mechanism of pyroptosis and other processes downstream of caspase 1 activation. Identification of proteins cleaved by caspase 1 *in vivo* will probably provide a great deal of insight and allow a more thorough mechanistic description of this process. The localization or composition of the inflammasome could have some role in regulating protein processing by caspase 1. It remains to be determined whether the inflammasome complex can determine the fate of cells that have active caspase 1. Importantly, the physiological features downstream of caspase 1 activation, including pyroptosis, are conserved responses to multiple stimuli. Pyroptosis and other caspase 1-dependent processes are therefore relevant to our understanding of important beneficial host responses as well as medical conditions for which inflammation is central to the pathophysiology of disease.

DATABASES

Entrez Genome Project

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj

Anaplasma phagocytophilum | Bacillus anthracis | Candida albicans | Mycobacterium tuberculosis

UniProtKB

http://www.uniprot.org

ASC | caspase 1 | caspase 3 | caspase 6 | caspase 8 | caspase 9 | IFNγ | IL-1α | IL-1β | IL-6 | IL-8 | IL-18 | NAIP5 | NLRC4 | NLRP1b | NLRP3 | NOD1 | NOD2 | TNF | TIRAP

FURTHER INFORMATION

Brad T. Cookson's homepage

http://depts.washington.edu/mcb/facultyinfo.php?id=190

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Supplementary Material

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Abbreviations

Caspases	A group of cysteine proteases that, based on their physiological roles, can be divided into two groups: those involved in the initiation and execution of apoptosis (caspase 2, 3, 6, 7, 8, 9 and 10) and those that trigger inflammation (caspase 1 and related caspases).
Autophagy	A programme of cellular self-digestion in which cytoplasmic components are sequestered and degraded intracellularly in autophagosomes. Autophagic cell corpses are ultimately removed by phagocytosis.
Oncosis	A caspase-independent pathway of cell death triggered by exposure to toxins or physical damage that features organelle and cell swelling and culminates in cell lysis with release of intracellular contents that stimulate inflammatory responses.
Toll-like receptor	A transmembrane protein that contains a leucine-rich repeat domain and mediates host recognition of pathogen- and danger-associated molecular patterns located in the extracellular milieu or within endosomes.
Nod-like receptor	A protein that contains a leucine-rich repeat domain and mediates host recognition of pathogen- and danger-associated molecular patterns in the host cell cytosol.
Proteasome	A multiprotein complex that recognizes and degrades polyubiquitinated substrates.

Pyronecrosis	Results from *Shigella* infection at high MOI (multiplicity of infection), morphologically resembles oncosis and is NLRP3-dependent and caspase 1-independent.
Microvesicle	A membrane vesicle of less than 0.5 μm in diameter that is shed from the plasma membrane of eukaryotic cells.
Necrosis	Does not indicate a specific pathway of cell death, but is a post-mortem description of dead cells that have reached equilibrium with their surroundings.

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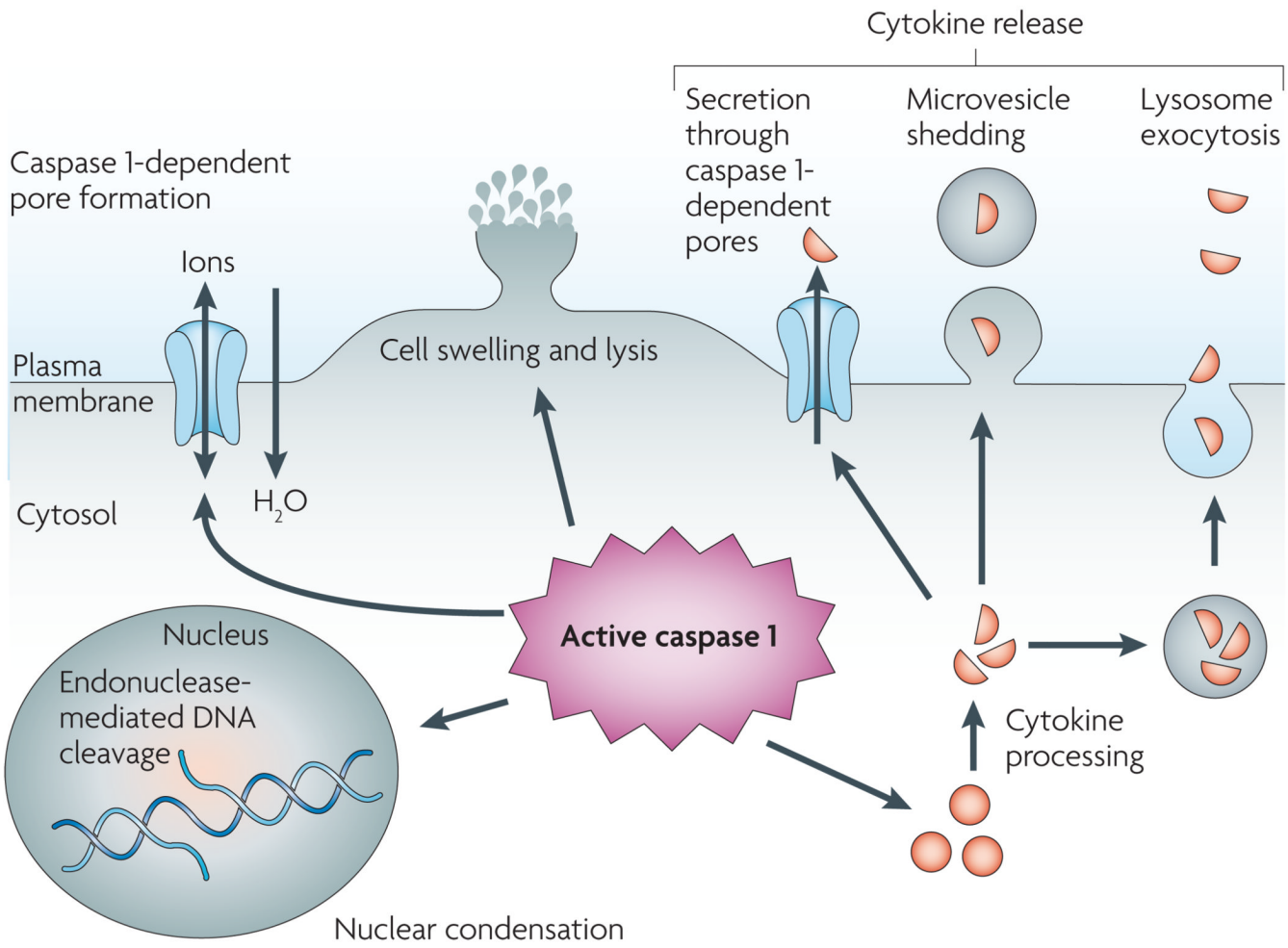

Figure 1. Pyroptosis, an inflammatory host response

Caspase 1 is cleaved and activated in response to multiple stimuli, but once activated, caspase 1 results in a conserved programme of cell death referred to as pyroptosis. Caspase 1 activation also leads to rapid formation of plasma-membrane pores with a diameter of 1.1–2.4 nm. These pores dissipate cellular ionic gradients, allowing water influx, cell swelling and osmotic lysis. The pro-forms of interleukin-1β (IL-1β) and IL-18 are processed by caspase 1 and released during pyroptosis, although the exact mechanism of secretion remains controversial. Secretion does not require lysis and is temporally associated with caspase 1-dependent pore formation, suggesting that these pores facilitate cytokine release. Other suggested secretion mechanisms include caspase 1-independent lysosome exocytosis and microvesicle shedding. Caspase 1 activity results in cleavage of chromosomal DNA by an unidentified endonuclease. Cleavage of DNA does not result in the oligonucleosomal fragments observed during apoptosis. Nuclear condensation is also observed but nuclear integrity is maintained, unlike the nuclear fragmentation observed during apoptosis.

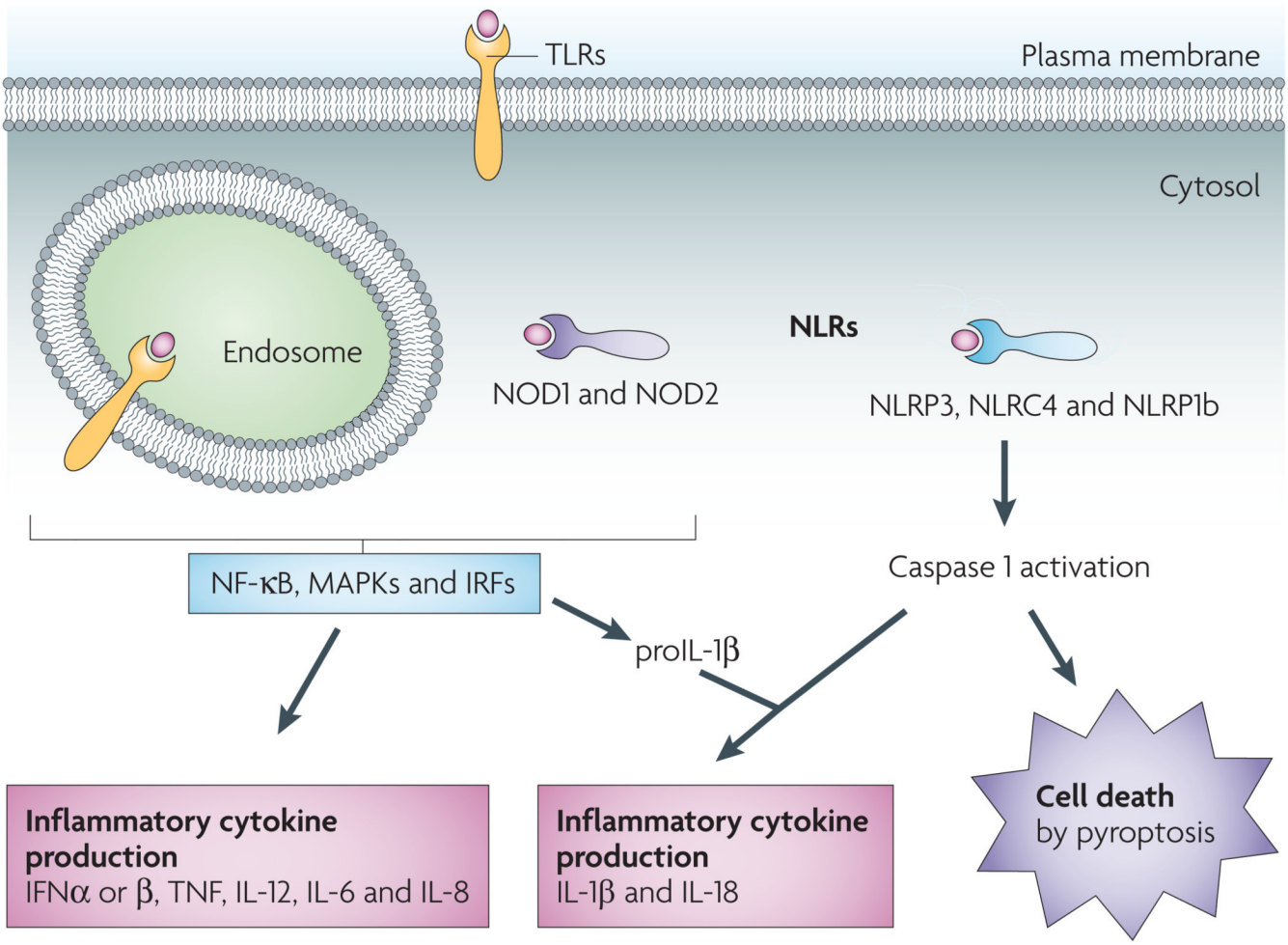

Figure 2. Sensing of host- and microorganism-derived 'danger' signals leads to two distinct outcomes: cellular activation and cell death

Leucine-rich repeat (LRR) domains mediate host recognition of pathogen- and danger-associated molecular patterns. Toll-like receptors (TLRs) are LRR-containing transmembrane proteins that detect danger signals located in the extracellular milieu and within endosomes. TLRs initiate a signalling cascade that leads to cellular activation (through nuclear factor-κB (NF-κB)-, mitogen-activated protein kinase (MAPK)- and interferon (IFN)-regulatory factor (IRF)-dependent pathways) and inflammatory cytokine production (including IFNα, IFNβ, tumour necrosis factor (TNF), interleukin-12 (IL-12), IL-6, IL-8 and pro-IL-1β). Nod-like receptors (NLRs) function in the recognition of danger signals introduced into the host cell cytosol. Like TLRs, NOD1 (nucleotide-binding oligomerization domain-containing protein 1) and NOD2 stimulation results in cytokine production. Another subset of NLRs mediate activation of the cysteine protease caspase 1, which triggers caspase 1-dependent cell death (pyroptosis) and processing and release of the inflammatory cytokines IL-18 and IL-1β. NLRC4, NLR family CARD domain-containing protein 4; NLRP3, NACHT, LRR and PYD domains-containing protein 3. NLRP1b, NAHCT, LRR and PYD domains-containing protein 1b.

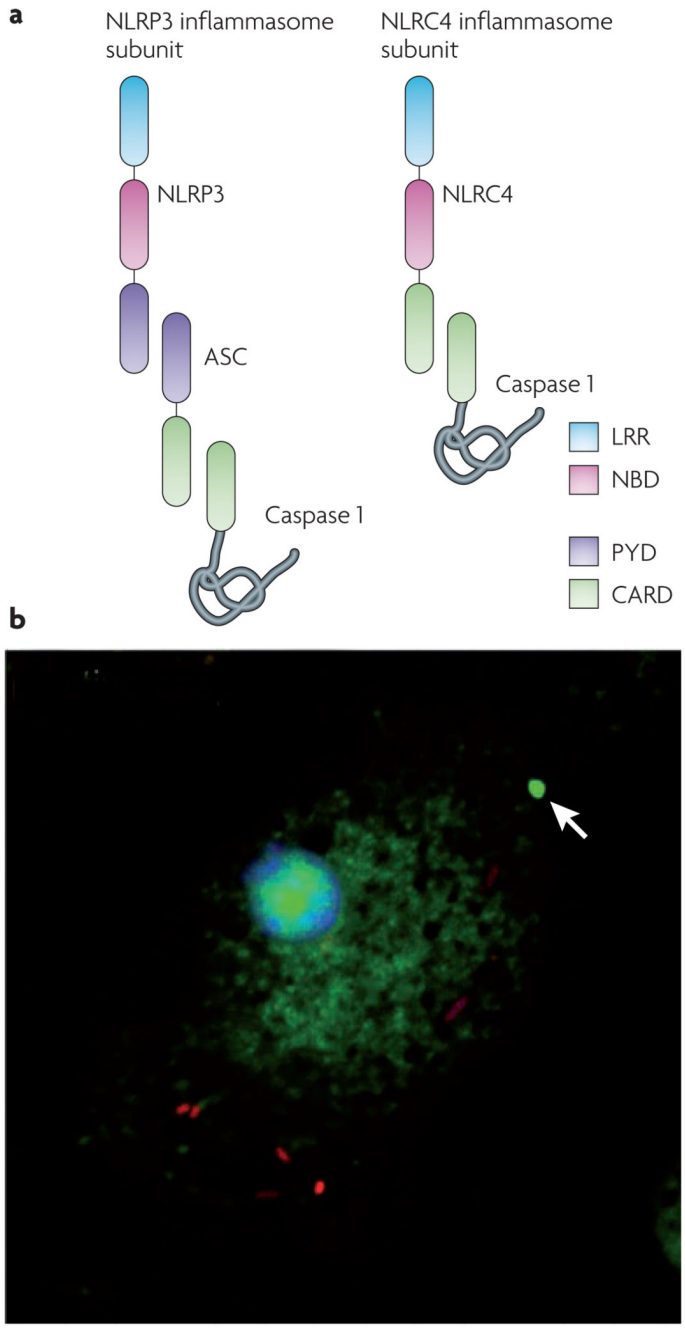

Figure 3. Components of the inflammasome and visualizing the inflammasome complex

a | Nod-like receptor (NLR) leucine-rich repeat (LRR) domains are implicated in sensing a range of intracellular 'danger' signals. After ligand recognition, the nucleotide-binding and oligomerization domain (NBD) mediates nucleotide-dependent self-association of NLRs. Some NLRs, such as NLRP3 (NACHT, LRR and PYD domains-containing protein 3; also called NALP3), contain a pyrin (PYD) domain that interacts with the adapter protein ASC (apoptosis-associated speck-like protein containing a CARD). ASC contains a caspase activation and recruitment domain (CARD) that binds and facilitates activation of caspase 1. Other NLRs, such as NLRC4 (NLR family CARD domain-containing protein 4; also known as IPAF), contain a CARD and can directly interact with caspase 1. However, ASC is often

required for NLRC4-dependent caspase 1 activation, indicating that ASC may participate in NLRC4 inflammasome formation or play an additional part in caspase 1 activation. **b** | Salmonella (red) infection of macrophages results in activation of caspase 1 (green), which is visualized here using a fluorescently labelled inhibitor of the active enzyme. Active caspase 1 is often concentrated within a single focus (indicated by the arrow) and diffusely distributed throughout the cytoplasm. A similar distribution of active caspase 1 is seen in macrophages treated with Bacillus anthracis lethal toxin[20].

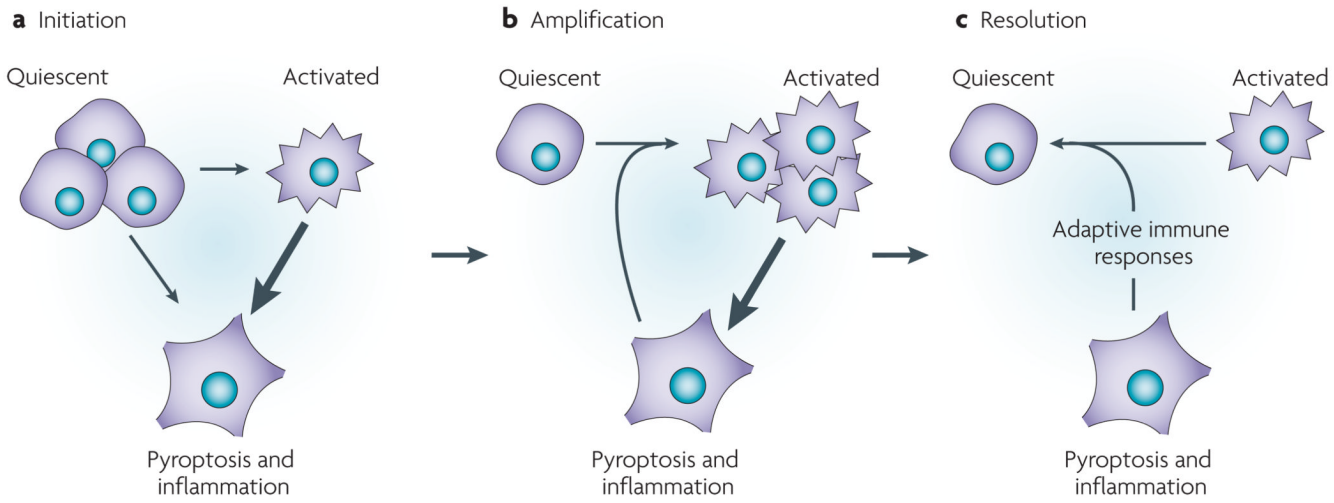

Figure 4. Caspase 1 activation in health and disease: fighting infection versus pathological inflammation

Caspase 1 plays a protective part in the response to microbial infection. **a** | In response to infection, quiescent cells undergo caspase 1 activation and pyroptosis, allowing cleavage and release of interleukin-18 (IL-18), IL-1β and other inflammatory intracellular contents. Quiescent cells can also undergo 'activation' in response to inflammatory mediators, thereby lowering the threshold for caspase 1 activation and pyroptosis and stimulating increased production of IL-1β. **b** | As infection progresses, the inflammation that occurs as a consequence of pyroptosis leads to an increased population of activated cells that are primed to undergo pyroptosis and have increased inflammatory potential. **c** | Inflammatory contents produced during pyroptosis recruit and activate immune cells and stimulate the development of adaptive immune responses. This contributes to the control and ultimate resolution of microbial infection, and returns tissues to their resting state. Alternatively, caspase 1 activation can be detrimental, as mutations in Nod-like receptor (NLR) proteins or the persistence of sterile inflammatory stimuli can result in inappropriate and/or excessive caspase 1 activation. The inflammation produced by this process increases the population of activated cells that are primed to undergo pyroptosis and express increased levels of IL-1β, and the amplification cycle persists (**b**). This potentiates the response and maintains an inflammatory state, which, if uninterrupted, leads to pathology.

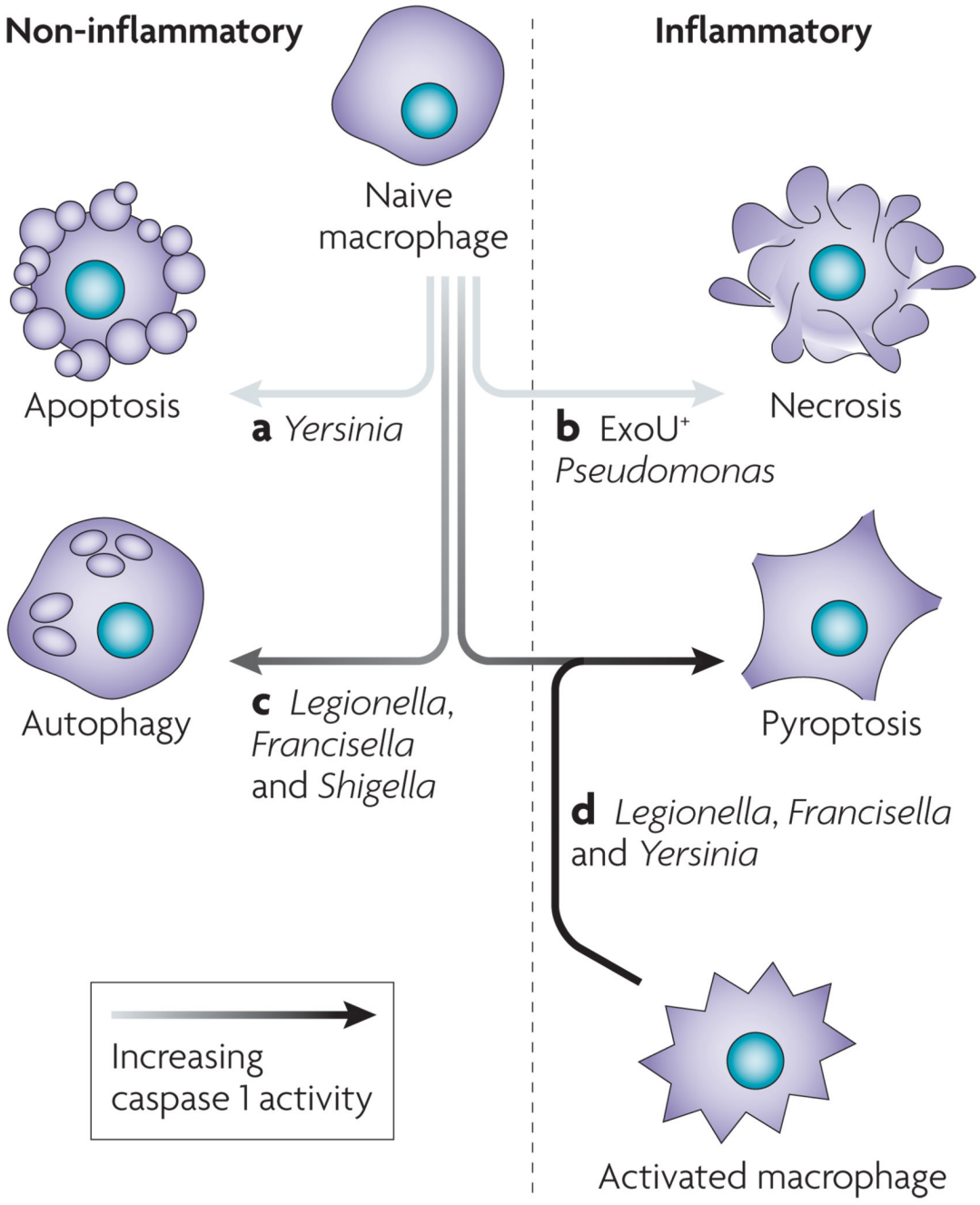

Figure 5. Susceptibility to pyroptosis is governed by pathogen and host modulation of caspase 1 activation

Pathogens have mechanisms for modulating cell death by inhibiting caspase 1 activation or inducing an alternative form of cell death that is more conducive to their continued replication. *Yersinia* (**a**) and *Pseudomonas* (**b**) translocate type III secretion effectors, resulting in apoptosis and necrosis, respectively. Pathogens can fail to induce robust caspase 1 activation owing to suboptimal 'danger' signal production by the pathogen (**c**). In addition, host mutations may not allow sufficient levels of caspase 1 activation to trigger pyroptosis. These infected macrophages often display features that are consistent with autophagy. Robust production of caspase 1-activating ligands by *Legionella* during infection of a

susceptible macrophage triggers pyroptosis (**d**). Not all cells are uniformly susceptible to pyroptosis, and macrophage activation enhances caspase 1 activation (FIG. 4) in response to *Yersinia* and *Francisella* infection, which do not stimulate pyroptosis in naive macrophages.