PYROPTOSIS

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Introduction

- **Pyroptosis : caspase 1-dependent cell death**, an inherently **inflammatory process of cell death,**
  - triggered by various pathological stimuli,
    - stroke, heart attack or cancer, and for controlling microbial infections.

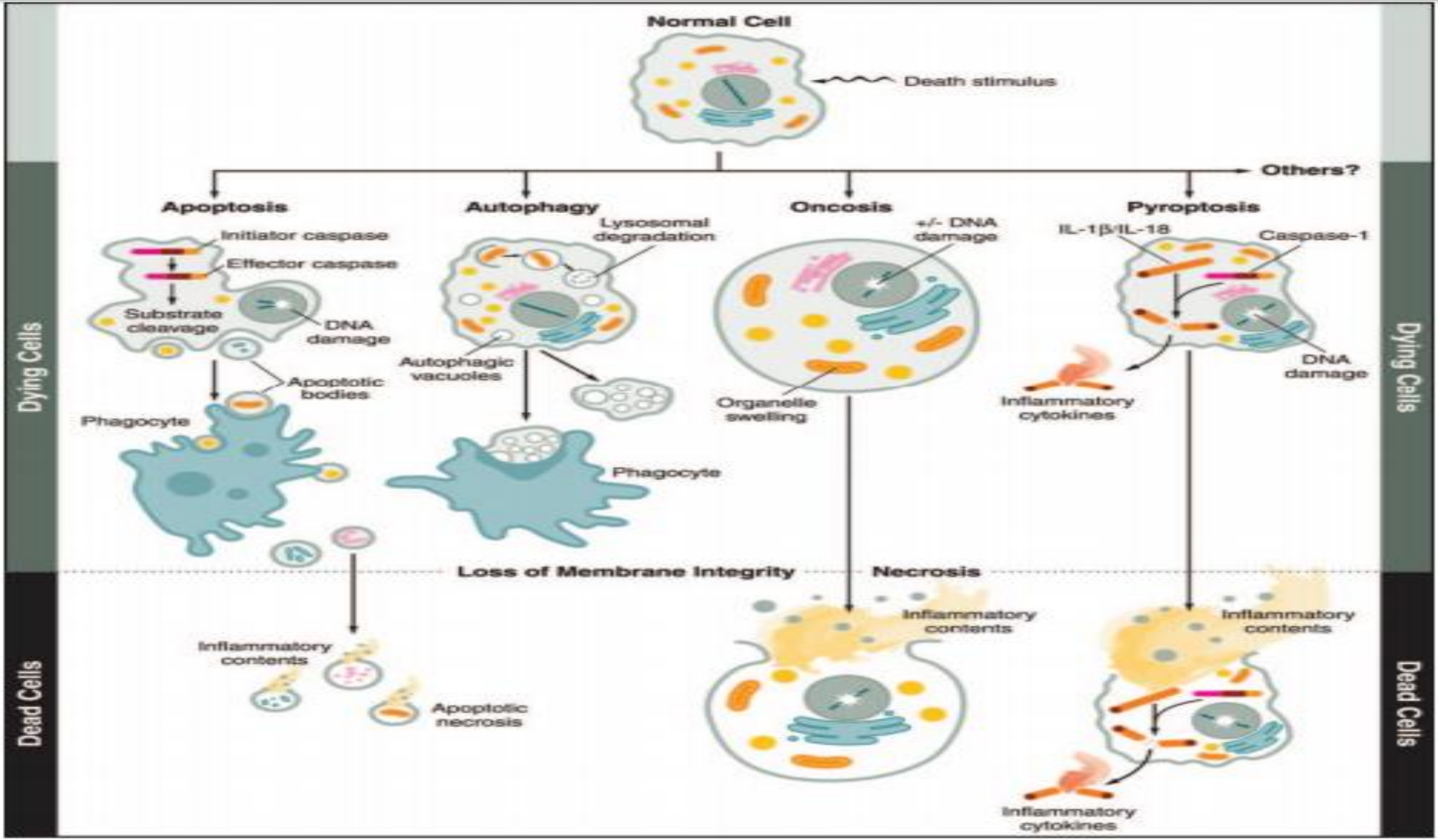
- In Greek ‘pyro’, relating to fire or fever, and

  ‘ptosis’, meaning a falling

- Pathogens have evolved mechanisms to inhibit pyroptosis,
  - there is a competition between host and pathogen to regulate pyroptosis, and the outcome dictates life or death of the host.

TABLE 1. Relevant terms for describing dead and dying cells

Term	Characteristic(s)
Programmed cell death	Dependent on genetically encoded signals or activities within the dying cell; a sequence of potentially modifiable events leading to the death of the cell
Apoptosis	Mediated by a subset of caspases (Fig. 1); morphology includes nuclear and cytoplasmic condensation and formation of membrane-bound cellular fragments or apoptotic bodies; not inflammatory
Autophagy	Degradation of cellular components within the dying cell in autophagic vacuoles; not inflammatory
Oncosis	Prelethal pathway leading to cell death accompanied by cellular and organelle swelling and increased membrane permeability; proinflammatory
Pyroptosis	Proinflammatory pathway resulting from caspase-1 activity leading to membrane breakdown and proinflammatory cytokine processing
Necrosis	Postmortem observation of dead cells that have come into equilibrium with their environment


Normal Cell
Death stimulus
Others?
Apoptosis
Autophagy
Oncosis
Pyroptosis
Initiator caspase
Effector caspase
Substrate cleavage
DNA damage
Apoptotic bodies
Phagocyte
Lysosomal degradation
Autophagic vacuoles
Phagocyte
+/- DNA damage
Organelle swelling
IL-1β/IL-18
Caspase-1
DNA damage
Inflammatory cytokines
Dying Cells
Loss of Membrane Integrity
Necrosis
Dead Cells
Inflammatory contents
Apoptotic necrosis
Inflammatory contents
Inflammatory cytokines

Caspase 1

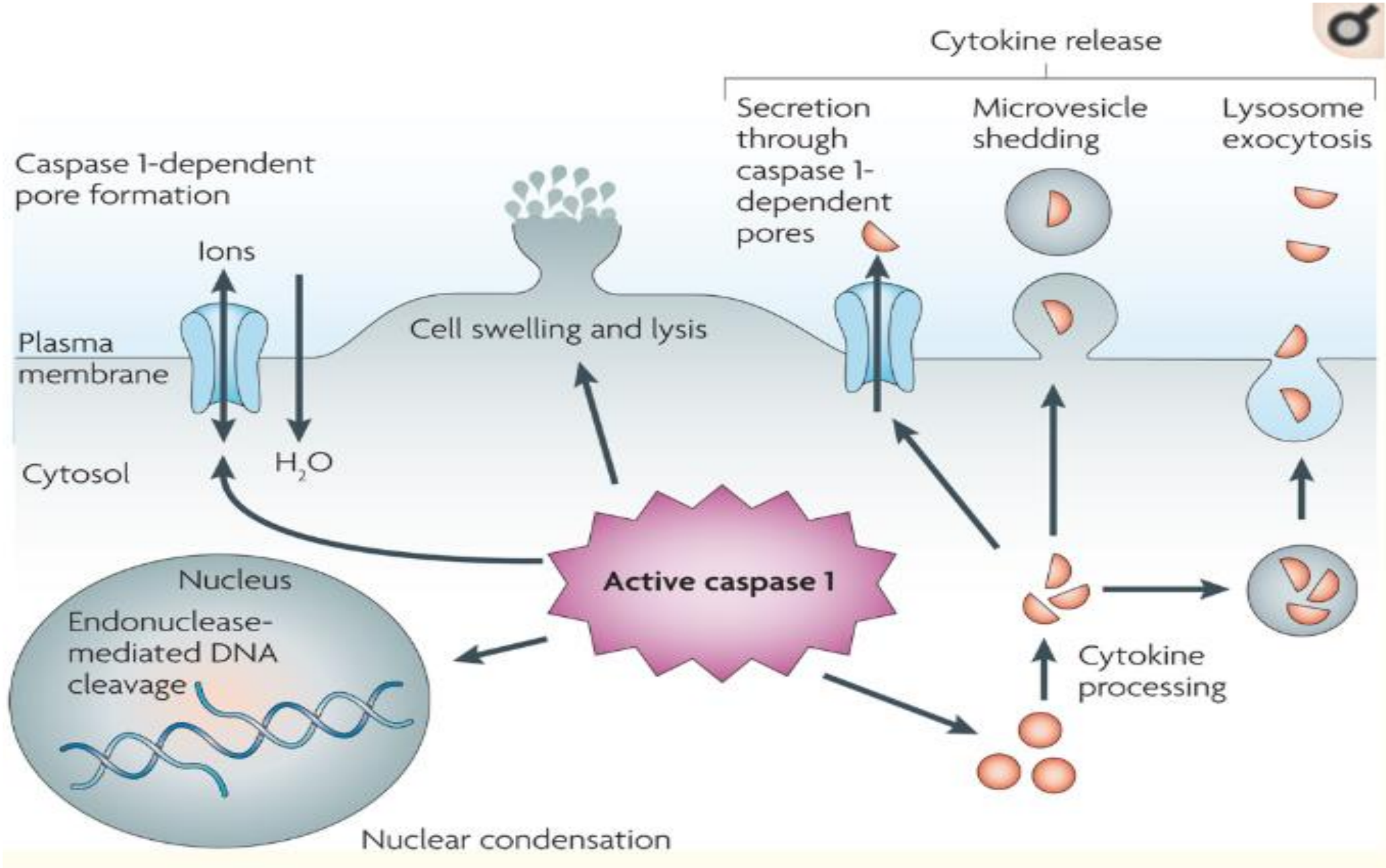
- Caspase 1 : a protease
  - interleukin IL-1β-converting enzyme
  - processes the inactive precursors of interleukin 1β (IL-1β) and IL-18 into mature inflammatory cytokines
  - rapid cell death characterized by plasma-membrane rupture and release of proinflammatory intracellular contents.

- However, the mechanism, characteristics and outcome of caspase 1-dependent cell death are distinct from apoptosis.

Pyroptosis, an inflammatory host response

Activated, caspase 1 leads to

1. 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.
2. Secretion through caspase 1-dependent pore formation :
   - these pores facilitate cytokine release.
3. microvesicle shedding
4. lysosome exocytosis


Cytokine release
Secretion through caspase 1-dependent pores
Microvesicle shedding
Lysosome exocytosis
Caspase 1-dependent pore formation
Ions
Plasma membrane
Cytosol
H_2O
Cell swelling and lysis
Active caspase 1
Nucleus
Endonuclease-mediated DNA cleavage
Nuclear condensation
Cytokine processing

- Caspase 1 activity also 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.

- 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.
- ICAD degradation does not occur during pyroptosis.
  - instead results from the activity of an unidentified caspase 1-activated nuclease

Pyroptosis vs apoptosis

- Caspase 1 is not involved in apoptosis, and caspase 1-deficient mice have no defects in apoptosis and develop normally

- The apoptotic caspases, including caspase 3, caspase 6 and caspase 8, are not involved in pyroptosis and
  - substrates of apoptotic caspases, including poly (ADP-ribose) polymerase and
  - inhibitor of caspase-activated DNase (ICAD),

  do not undergo proteolysis during pyroptosis

- Furthermore, loss of mitochondrial integrity and release of cytochrome *c*, which can activate apoptotic caspases, do not occur during pyroptosis

- 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

Factors that are involved in the pathway….

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’

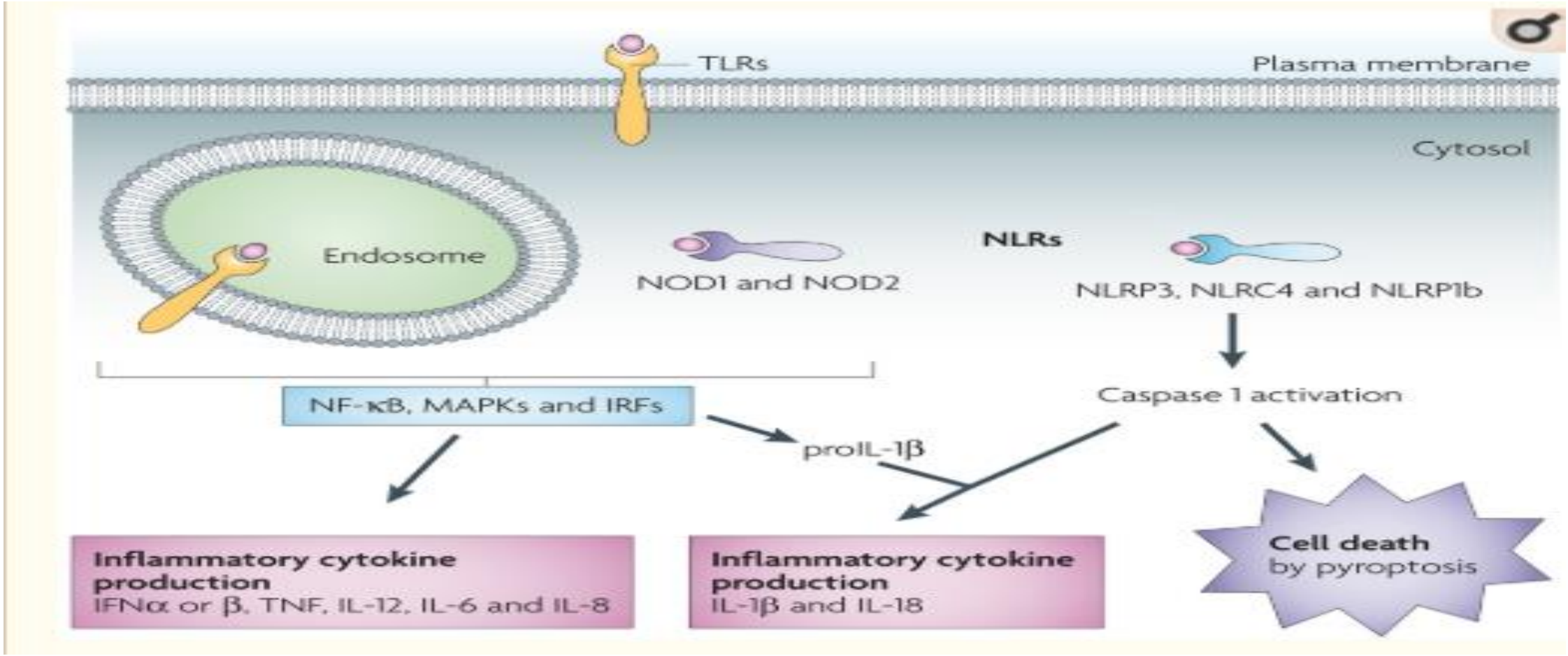
- Toll-like receptors (TLRs)-

  initiate a signalling cascade, leads to cellular activation and production of inflammatory cytokines, eg. TNF , IL-6, IL-8 and type I interferons (IFNs), in response to extracellular signals.

- Nod-like receptors (NLRs):

  function in the recognition of danger signals introduced into the host cell cytosol.

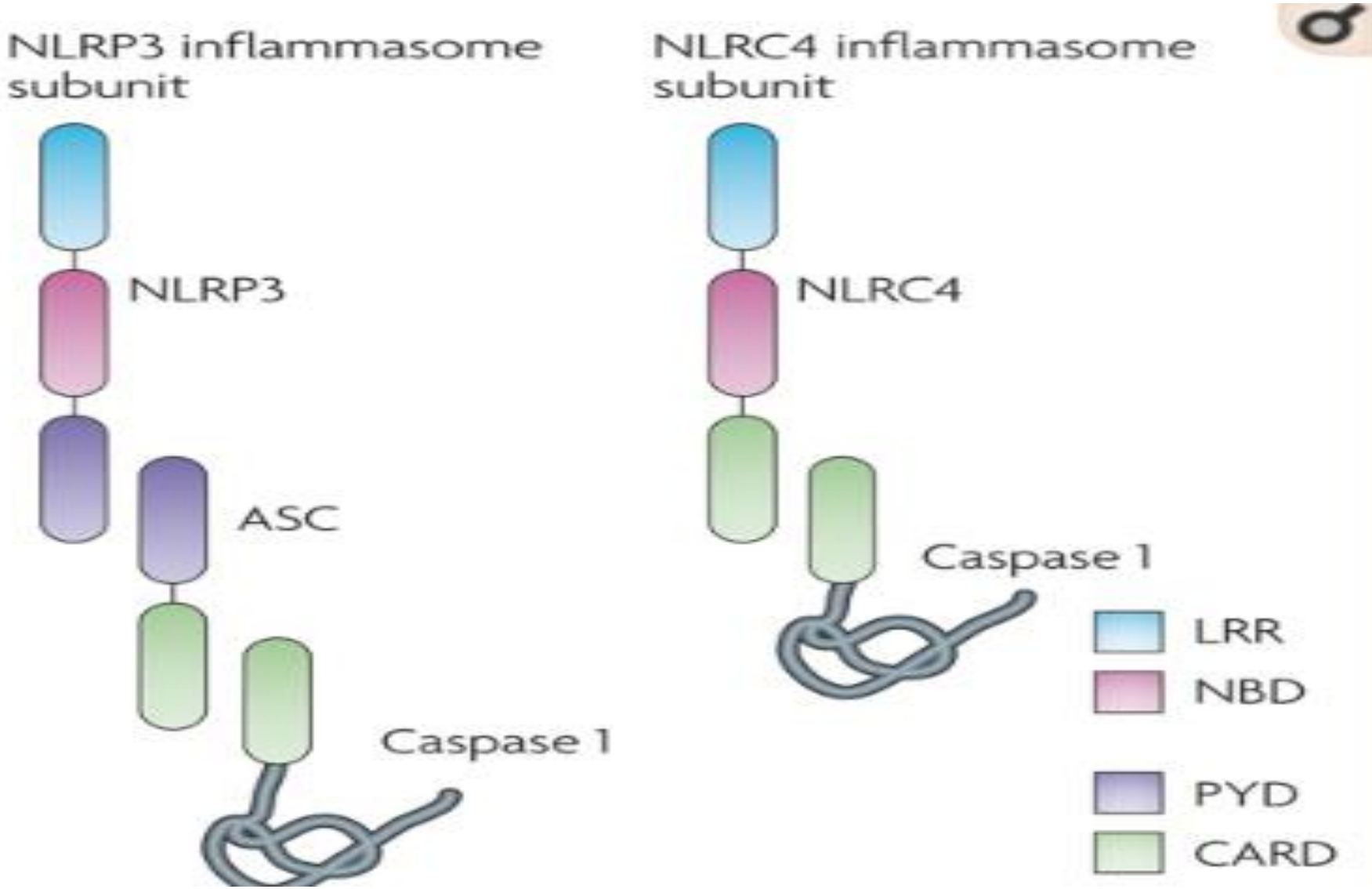
- Thus, TLRs and NOD1 and NOD2 prime cells to undergo caspase 1 activation



- 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).


NLRP3 inflammasome subunit
NLRP3
ASC
Caspase 1
NLRC4 inflammasome subunit
NLRC4
Caspase 1
LRR
NBD
PYD
CARD

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 responds to multiple stimuli, including pore-forming toxins, extracellular ATP in the presence of various pathogen-associated molecules, uric acid crystals, virus-associated DNA, RNA, asbestos and ultraviolet B irradiation.

- 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 activation.

- However, potassium efflux alone does not seem to be sufficient to trigger activation of caspase

- 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 activation.

- The NLR protein NLRC4 -
  - mediates the recognition of diverse bacterial pathogens, which during infection reside extracellularly or intracellularly
  - 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.

- introducing flagellin into the host cell, where its recognition is facilitated by NLRC4 .

- 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 .

- 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 involved.

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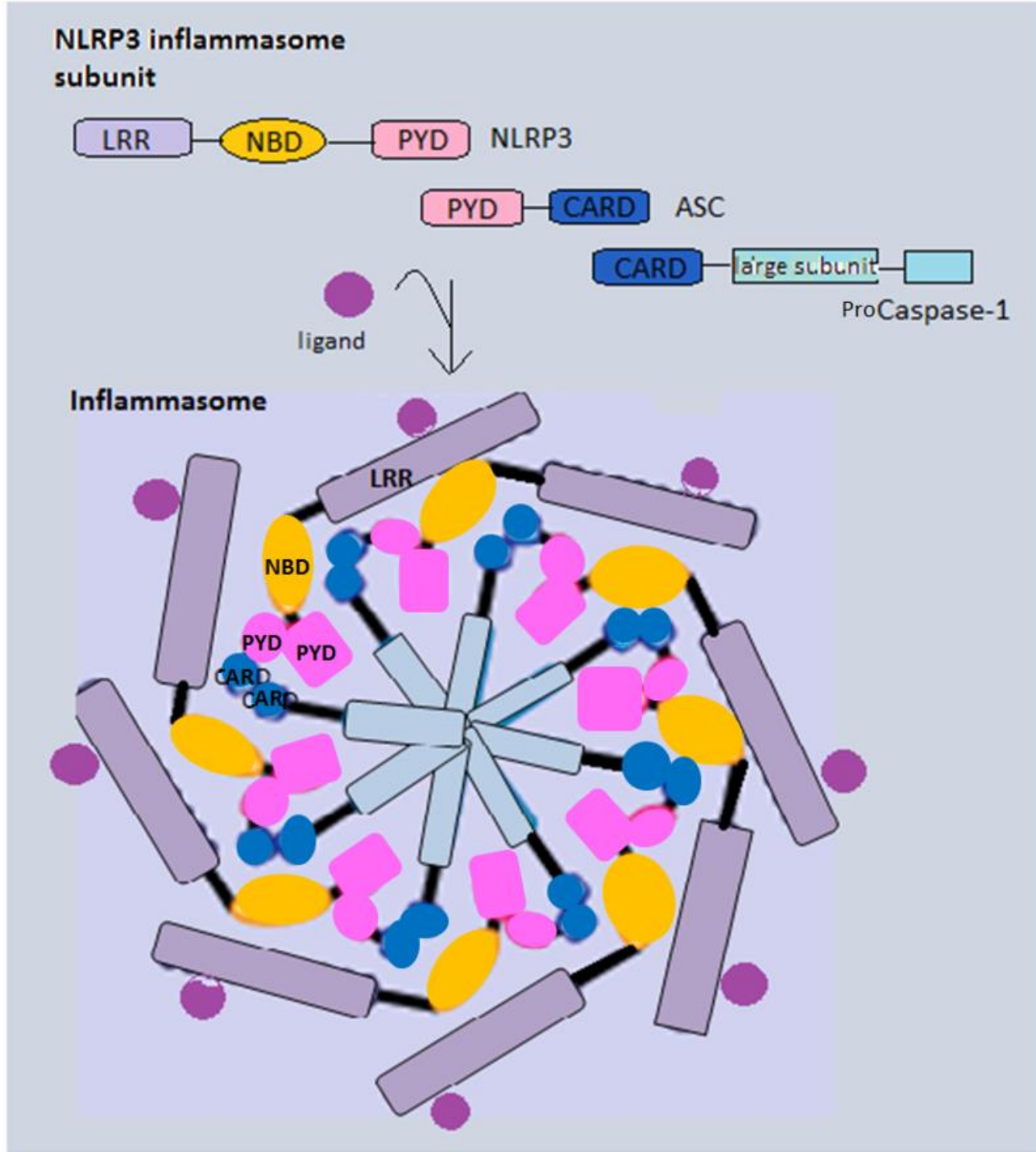
The inflammosome

- NLRs recognize their cognate host- or microorganism-derived danger signals and trigger formation of a multiprotein complex called the inflammasome, which contains caspase1.

- NLRs that have encountered their signal undergo nucleotide-dependent oligomerization using their nucleotide-binding domain.

- Some NLRs bind to the adapter protein ASC, which contains a caspase activation and recruitment domain (CARD) and interacts with caspase 1 .


NLRP3 inflammasome subunit
LRR
NBD
PYD
NLRP3
PYD
CARD
ASC
CARD
large subunit
ProCaspase-1
ligand
Inflammasome
LRR
NBD
PYD
PYD
CARD
CARD

- 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.

- The adapter protein ASC can self-associate and form similarly sized complexes in the absence of an NLR

- ASC facilitates caspase 1 activation even though it is not absolutely required for the binding of NLRC4 to caspase 1.

- Inflammasome components can also interact with proteins that activate alternative cellular processes or forms of cell death.

- 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.

Caspase 1-dependent processes

- IL-1β and IL-18 processing and secretion
- Additional inflammatory cytokines eg. TNF, IL6
- Inhibiting growth of intracellular bacteria
- Cell repair and survival

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.

- IL-18 induces IFNγ production and is important for the activation of T cells, macrophages and other cell types.

- Both IL-1β and IL-18 play crucial parts in the pathogenesis of a range of inflammatory and autoimmune diseases.

- Although neither cytokine is required for the process of cell death , their production contributes to the inflammatory response elicited by cells undergoing pyroptosis.

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.

- 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.

- Caspase 1 activity also enhances the killing of mycobacteria by stimulating trafficking of the bacteria to lysosomal compartments.

- However, caspase 1 is not required for the degradation of all bacteria.

Cell repair and survival

- Caspase 1 activation fails to trigger pyroptosis in all cell types, Epithelial cells use caspase 1 activation to prevent cell death.

- Caspase 1 activation stimulates lipid production and membrane repair in response to the pore-forming toxins aerolysin and α-toxin, and inhibition of caspase 1 activity actually enhances cell lysis.

- 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.

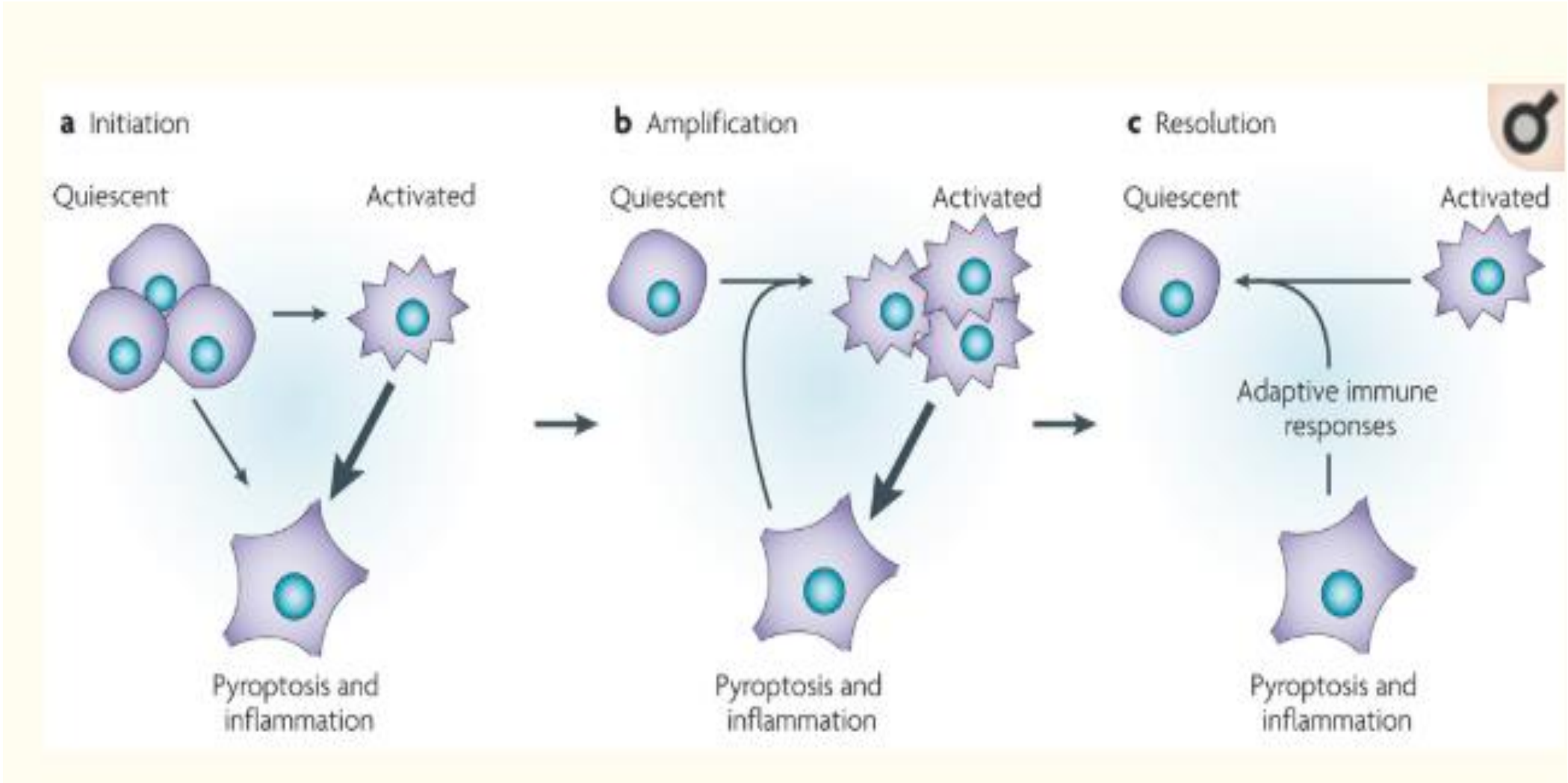
- The magnitude of caspase 1 activation modulates the response to microbial stimuli and host factors that warrant an inflammatory response.

- 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.

Caspase 1 in host response & 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 .
- Mutations in NLR proteins can lead to inappropriate caspase 1 activation, which is associated with hereditary autoinflammatory syndromes.
- Caspase 1 is involved in the pathogenesis of several diseases, including myocardial infarction , cerebral ischaemia, inflammatory bowel disease, neurodegenerative diseases and endotoxic shock.
- 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.



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

- 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.

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.

Host cell activation redirects cell death

- Caspase 1 activation clearly functions as a host defence mechanism in a wide range of microbial infections.

- caspase 1 activation limits pathogen replication, enhances innate and adaptive immune responses, and improves host survival

SUMMARY

	Characteristics	Apoptosis	Pyroptosis	Necrosis
Morphology	Cell lysis	NO	YES	YES
	Cell swelling	NO	YES	YES
	Pore formation	NO	YES	YES
	Membrane blebbing	YES	NO	NO
	DNA fragmentation	YES	YES	YES
Mechanism	Caspase-1	NO	YES	NO
	Caspase-3	YES	NO	NO
	Cytochome-c release	YES	NO	NO
Outcome	Inflammation	NO(anti)	YES	YES
	Programmed cell death	YES	YES	NO

Pyroptosis in Disease

- plays an important part in host defenses against infection
- linked to neurodegenerative diseases like Alzheimer’s, as high levels of caspase-1 have been found in brains from Alzheimer’s patients.
- In cardiovascular disease, NLRP3 mediated pyroptosis, in response to cholesterol or reactive oxygen species in endothelial cells, leads to cell death and destruction of vascular endothelial function.
- In some cancers, carcinogenic changes can lead to inflammatory responses through pyroptosis, which promotes tumor progression and caspase-1, has been found to be inhibited in prostate and hepatocellular carcinoma, preventing cell death