Mechanisms of Pyroptosis

By

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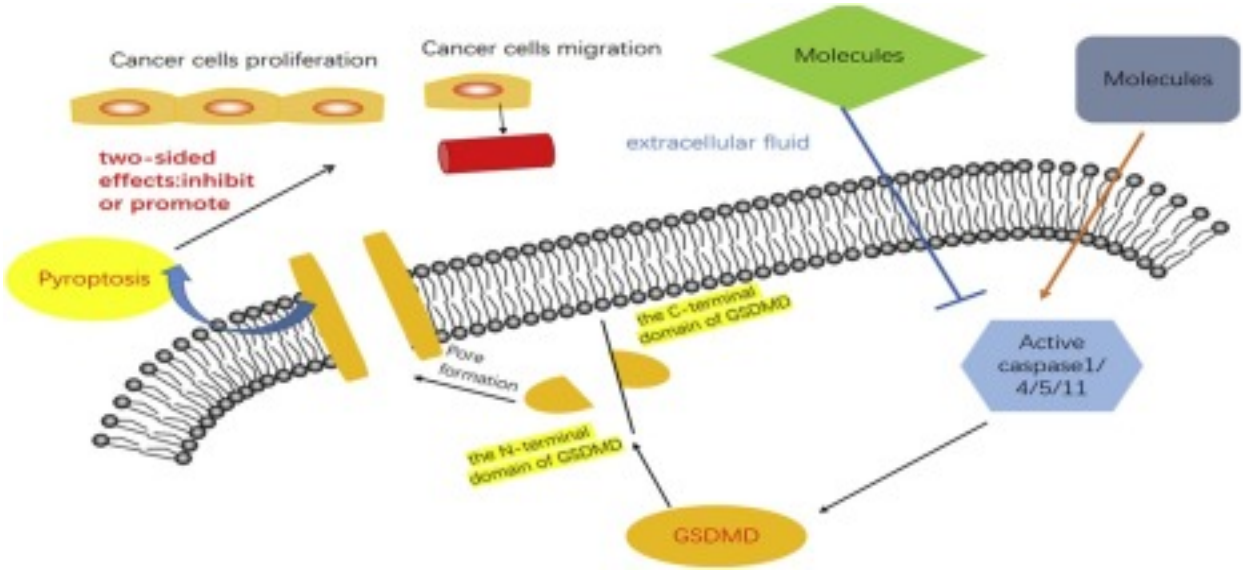


Pyroptosis

Introduction to Pyroptosis

- Pyroptosis is a form of inflammatory programmed cell death, triggered by various pathological stimuli such as stroke, heart attack, cancer and microbial infections.
- Other known types of programmed cell death include apoptosis and necroptosis.

Pyroptosis

- Pyroptosis is fundamentally distinct from other cell death pathways by its exclusive dependency on caspase-1.
- Caspase-1 is a principle effector protease within the pyroptotic cell death pathway.

- Pyroptosis promotes the rapid clearance of various bacterial and viral infections by removing intracellular replication niches and by enhancing the host's defensive response.
- Cells can use a broad range of intracellular and extracellular mechanisms for detecting different "danger" signals generated or released during infection or injury.

- These detection mechanisms typically involve:

- pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs).

- NOD-like receptors (NLRs).

- RIG-I-like receptors (RLRs).

- Absent in melanoma 2 (AIM2).

PRRs initiate a signalling cascade that leads to activation and production of inflammatory cytokines upon recognition of (pathogen associated molecular patterns or PAMPs, or danger associated molecular patterns or DAMPS, respectively).

Recognition of PAMPs, DAMPs, and some foreign toxins can lead to inflammasome activation, which triggers activation of caspase-1, and the initiation of pyroptosis.

Cell surface-associated TLRs typically recognize microbial membrane components (such as the lipid A component from LPS, peptidoglycans, mannan, etc.), while intracellular TLRs recognize bacterial and viral nucleic acids, as well as some "self" nucleic acids present during particular disease states.

In addition to promoting an immune response, activation of the inflammasome also leads to an increase in pyroptotic cell death. Some examples of pyroptosis include the death of salmonella-infected macrophages and T helper (Th) cells that die as a result of abortive HIV infection. In addition, pyroptosis is stimulated by non-infectious stimuli, including host factors produced during myocardial infarction.

- **Lysis Through Cell Swelling**

  Caspase-1-regulated cellular 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.

- **DNA Cleavage**

  Occurs during pyroptosis, but lacks the nuclear integrity and oligonucleosomal DNA fragmentation patterns characteristic of apoptosis.

- **Inflammasome**
- The inflammasome is a cytosolic multimeric signaling complex that coordinates the activation of an immune response against invading pathogens. Activation of the inflammasome subsequently leads to processing and activation of caspase-1.
- Once activated, caspase-1 cleaves pro-IL-1β and pro-IL-18 into their mature forms, and it cleaves Gasdermin D (encoded by GSDMD) to induce pore opening and pyroptosis. The cytosolic pathogen recognition receptors capable of forming inflammasome complexes include NLRs, ALRs, and the tripartite motif (TRIM) family member Pyrin.

- **Release of Activated IL-1β and IL-18.**

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 the expression of a diverse array of cytokines and chemokines. IL-18 induces Interferon gamma (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.

- **The Signaling Cascades**
- TLR activation signals through the recruitment of specific adaptor molecules. Upon PAMP or DAMP recognition, TLRs recruit TIR-domain-containing adaptor proteins such as MyD88 and TRIF. MyD88 and TRIF then initiate signal transduction pathways that lead to the activation of NF-κB, IRFs, or MAP kinases, which subsequently regulate the expression of cytokines, chemokines, and type I IFN.

- **The Signaling Cascades**

- Cytosolic scaffolds can detect the presence of danger signals by directly detecting PAMPs or DAMPs, or indirectly detecting a secondary messenger. The scaffolds serve to recruit and oligomerize procaspase-1. Procaspase-1 recruitment can occur either directly or indirectly through the involvement of apoptosis-associated speck-like protein (ASC), which contains a caspase recruitment domain (CARD). Procaspase-1 is oligomerized into filaments that allow for its subsequent autoactivation. Active caspase-1 then functions to cleave pro-IL-1β and pro-IL-18, thus triggering their release into the extracellular space where they can exert an effect on immune cells, promoting both local and systemic immune responses.

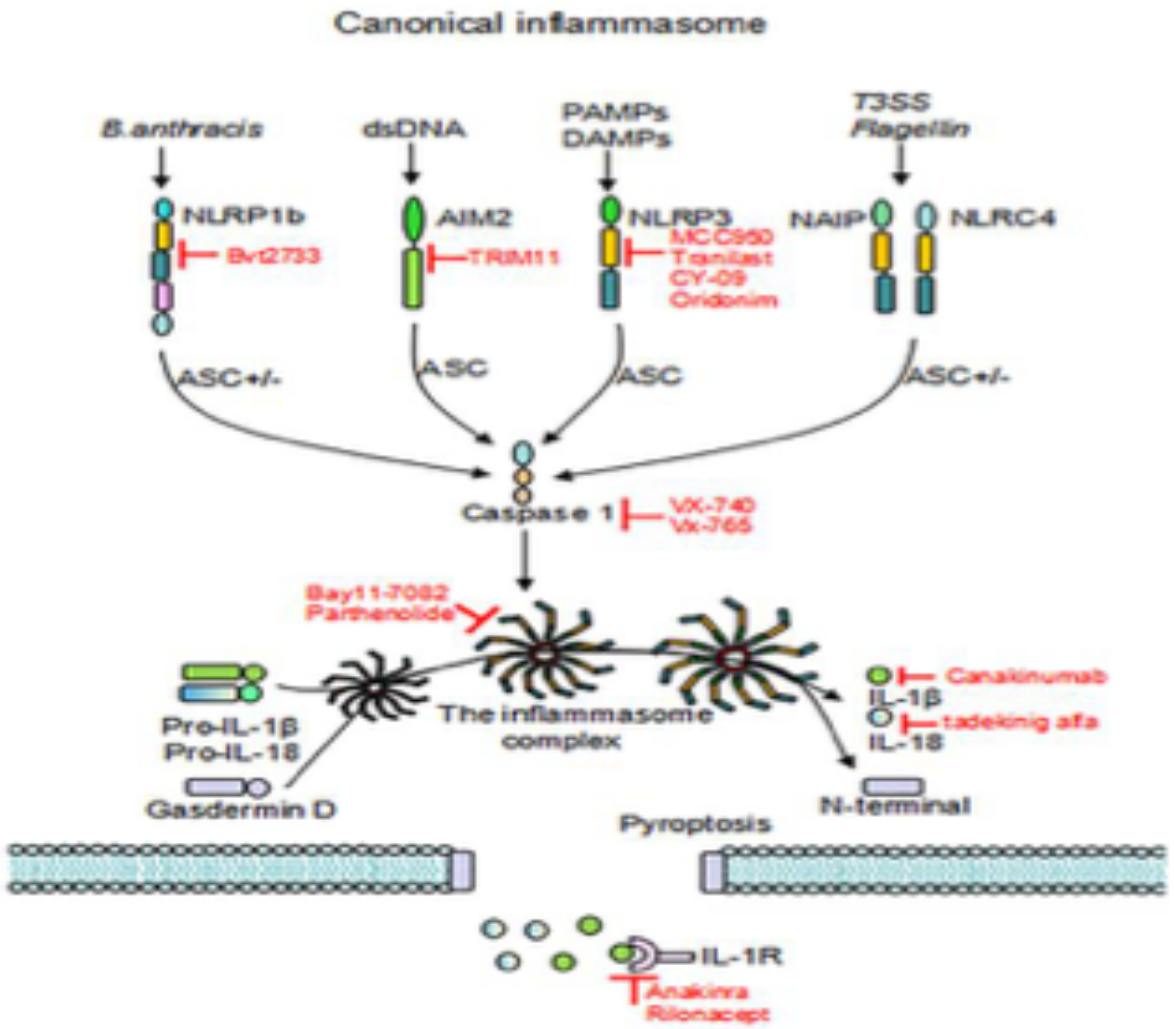
- **Clinical Aspects**

- Pyroptosis acts as a defense mechanism against infection by inducing inflammation. The formation of inflammasomes and the activity of caspase-1 determine the balance between eradication of the pathogen-associated disease state versus coping with a protracted infection. In a healthy cell, caspase-1 activation helps to fight infection. For example, caspase-1 induced pyroptotic cell death severely limits the internal spread of Salmonella and Shigella bacteria, via elimination of infected host cell incubators necessary for continued bacterial reproduction . When the danger signal is recognized, the infected quiescent cells become activated, undergoing pyroptosis and secreting inflammatory cytokines IL-1β and IL-18. IL-18 will stimulate IFNγ production and initiates the development of Th1 responses. Th1 responses tend to release cytokines that direct an immediate removal of the pathogen .

- **Clinical Aspects**

- Highly conserved cell death processes such as pyroptosis can and will function as a double edge sword. Inflammasome activation results in an increase in cytokine levels, which will augment the consequences of inflammation. This in turn, contributes to the development of the adaptive immune response as infection progresses. The ultimate resolution will clear pathogens. In contrast, persistent inflammation has also been linked to a variety of autoimmune and autoinflammatory diseases such as multiple sclerosis, Alzheimer's disease, Parkinson's disease, atherosclerosis, type 2 diabetes, and obesity. If the amplification cycles persist, metabolic disorder, auto-inflammatory diseases and liver injury associated with chronic inflammation will take place. Since pyroptosis is involved in many beneficial and detrimental inflammatory processes, this conserved cell death process remains an important area of continued study.

- **Clinical Aspects**
- ICT's **Pyroptosis/Caspase-1 Assays** utilize our popular FLICA® technology to detect caspase-1 activation. These kits contain the caspase-1 inhibitor reagents, which have the preferred binding sequence for caspase-1, TyrVal-Ala-Asp (YVAD).
- These kits also contain **Nigericin**, a potent microbial toxin derived from Streptomyces hygroscopicus, that acts as a potassium ionophore, capable of inducing a net decrease in intracellular levels of potassium which is crucial for oligomerization of the NLRP3 inflammasome and activation of caspase-1.


Canonical inflammasome
B.anthracis
dsDNA
PAMPs
DAMPs
T3SS
Flagellin
NLRP1b
Bvr2733
AIM2
TRIM11
NLRP3
MCC950
Tranilast
CY-09
Oridonin
NAIP
NLRC4
ASC+/-
ASC
ASC
ASC+/-
Caspase 1
VX-740
Vx-765
Bay11-7082
Parthenolide
Pro-IL-1β
Pro-IL-18
Gasdermin D
The inflammasome complex
Canakinumab
IL-1β
tadekinig afa
IL-18
N-terminal
Pyroptosis
IL-1R
Anakinra
Rilonacept

Thank you