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Popular Article

PYROPTOSIS: An introduction and overview in fish

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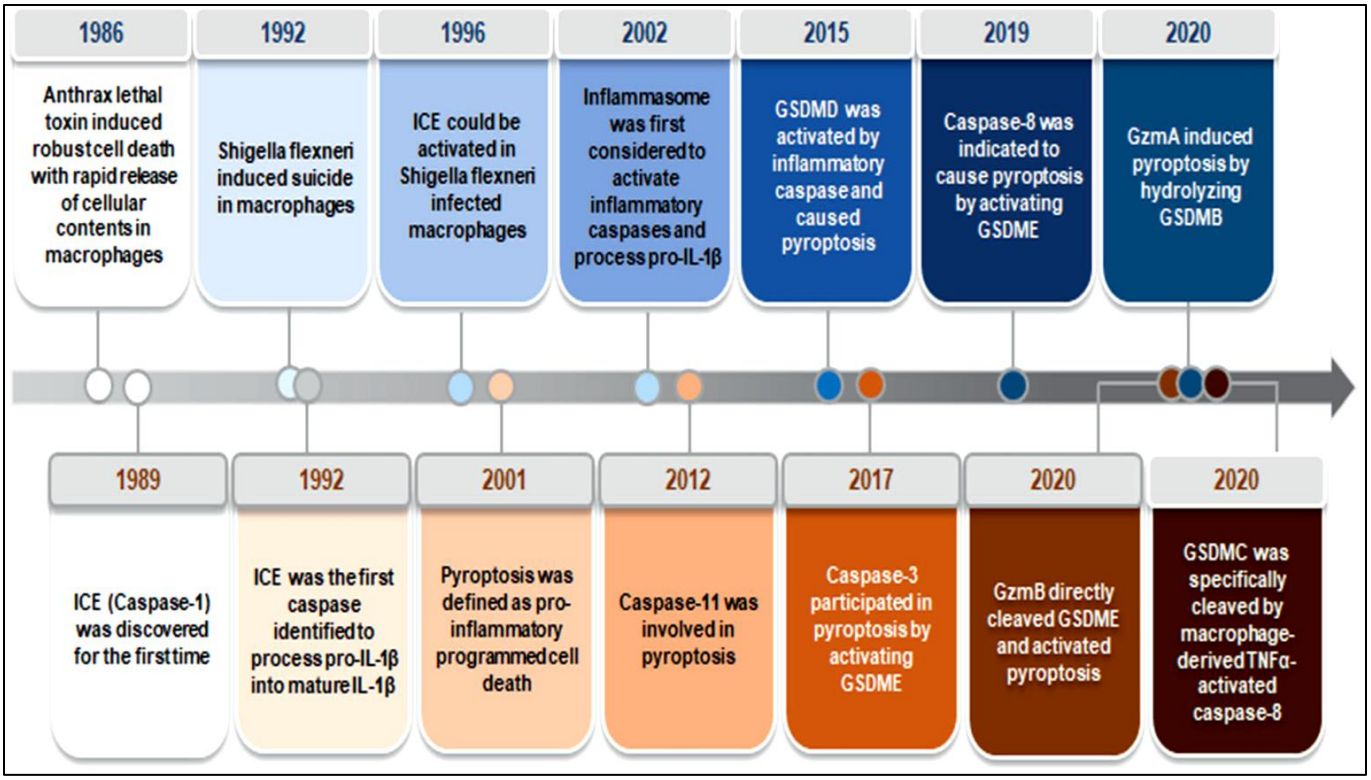
INTRODUCTION

Research on pyroptosis began in 1986 when Friedlander observed that anthrax lethal toxin (LT) treatment of primary mouse macrophages led to cell death and rapid release of cell contents. In 1989, Cerretti et al. and Thornberry et al. identified ICE (interleukin-1β-converting enzyme, caspase-1) as an inflammatory caspase. Zychlinsky et al. made the first observation of pyroptosis in 1992, noting its occurrence in Gram-negative bacterial pathogen Shigella-infected macrophages. Chen and colleagues (1996) discovered that Shigella flexneri invasion plasmid antigen B (ipaB) directly attaches to ICE, activating the enzyme within infected macrophages. Initially mistaken for apoptosis due to similarities such as caspase dependence, DNA damage, and nuclear condensation, this form of cell death was later recognized as distinct. The term ‘pyroptosis,’ derived from the Greek word’s ‘pyro’ (fire/fever) and ‘ptosis’ (falling), was coined to describe pro-inflammatory programmed cell death (D’Souza and Heitman, 2001).

The first theory of how an inflammatory caspase activates and processes pro-IL-1β was put out in 2002. Later, when the host was infected with Salmonella, Petr et al. discovered that non-canonical caspase-11 could cause cell death without depending on caspase-1. For a very long period, pyroptosis was thought to be caspase-1-induced monocyte death. Subsequently, it was shown that this

mechanism activated caspase-1 or caspase-11/4/5, cleaved gasdermin D (GSDMD), and allowed the N-terminal domain to oligomerize, creating gaps in the cell membrane that might cause the membrane to rupture. Diseases of the neurological system, infections, autoimmune disorders, cardiovascular disorders, and malignancies are intimately associated with pyroptosis.

Figure 1 : The timeline of pyroptosis discovery (Source: Yu et al., 2021)



GASDERMIN, THE EXECUTIONER OF PYROPTOSIS

In 2015, the definition of pyroptosis was established as gasdermin-mediated programmed death. Gasdermin A/B/C/D (GSDMA/B/C/D), gasdermin E (GSDME, sometimes called DFNA5), and DFNB59 (Pejvakin, PJVK) in humans (Gsdma1-3, Gsdmc1-4, Gsdmd, Dfna5, and Dfnb59 in mice) make up the gasdermin superfamily. GSDMD and DFNA5 are the two conserved proteins that have been researched the most in relation to pyroptotic mortality. All of these members—aside from Pejvakin—are made up of the N-terminal poreforming domain and the C-terminal repressor domain (PFD and RD), which are two conserved domains.

Pyroptosis may be induced by the PFD of most gasdermins; however, Pejvakin has not yet shown signs of this condition. Generally speaking, PFD and RD work together to sustain oligomerization in gasdermins, and RD may even reduce the cytotoxic effects of PFD. The N-terminal PFD separates from the C-terminal RD when the host is stimulated by a range of endogenous or exogenous factors. This causes the N-terminal PFD to oligomerize and create pores in the cell

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membrane, which releases inflammatory molecules and results in cell pyroptotic death. Gasdermins are cleaved by some caspases or granzymes.

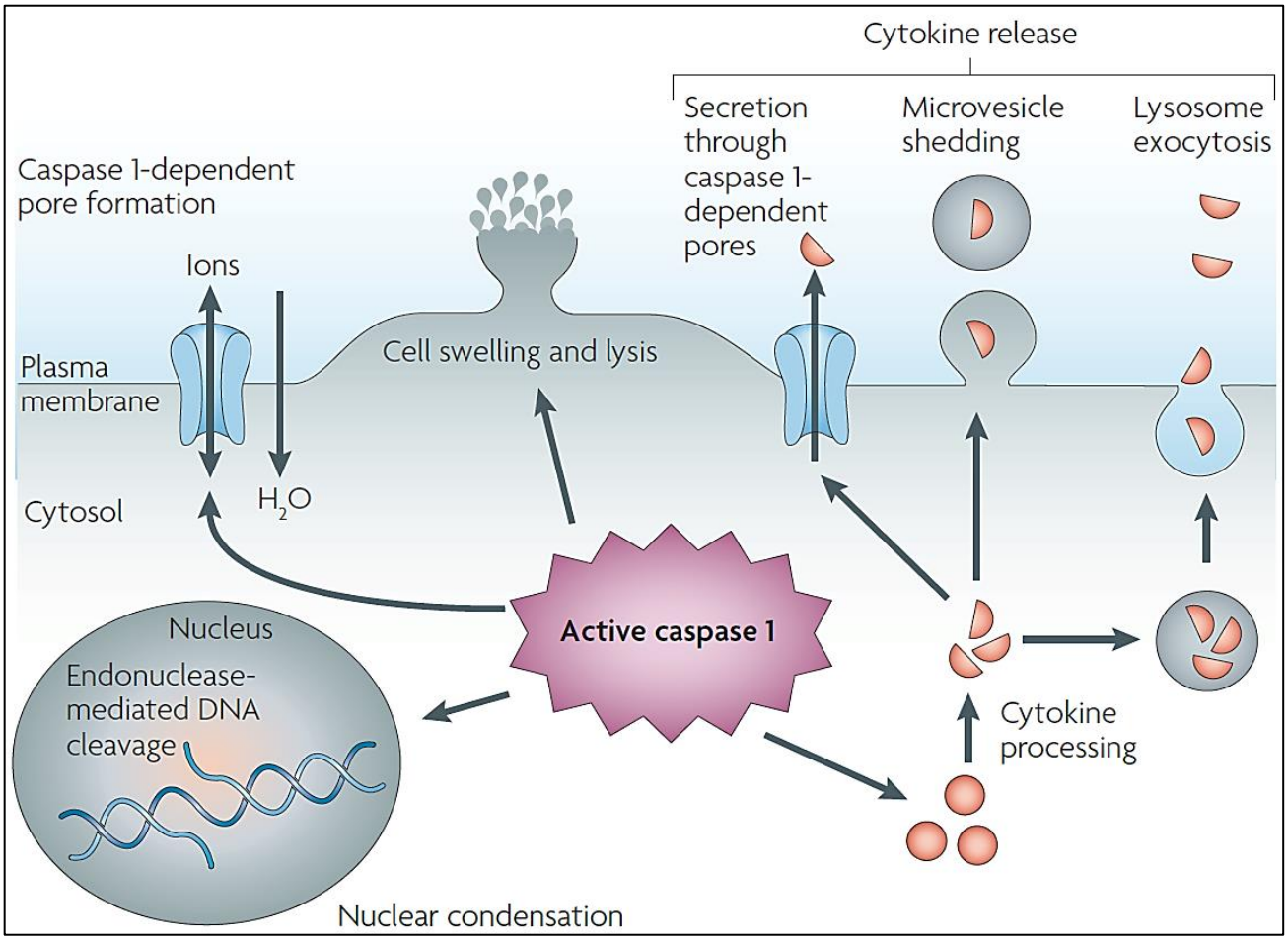

Figure 2: Pyroptosis, an inflammatory host response (Source: Bergsbaken et al., 2009)

THE CHARACTERISTICS OF PYROPTOSIS

Pyroptosis and apoptosis share several characteristics, such as chromatin condensation and DNA damage. It's interesting to note that before the cellular membrane bursts, pyroptotic cells emerged expanding and many bubble-like protrusions appeared on its surface. Comparably, membrane blebbing happens during apoptosis as well, and caspase-3 is required for this activity. But it is clear that pyroptosis differs from apoptosis in terms of its distinct morphological features. Although pyroptosis can result in inflammation when it is triggered by external or intracellular stimuli such bacteria, viruses, toxins, and chemotherapeutic medicines, it is widely accepted that apoptosis is a harmless kind of death.

In actuality, pyroptosis results in flattening of the cytoplasm leading to plasma membrane leaking, as opposed to the catastrophic rupture linked to necrosis. Furthermore, the activation of

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caspases or the release of granzymes causes the N terminal of gasdermin oligomerization and the formation of a pore (1-2 μm in diameter) in the plasma membrane, which permits the passage of mature IL-1β/IL-18, which has a diameter of 4.5 nm, and caspase-1, which has a diameter of 7.5 nm. Meanwhile, the water that passes through the holes promotes osmotic lysis and swelling of the cells, which breaks the plasma membrane and releases IL-1β and IL-18. Because of their low molecular weight, 7-aminoactinomycin (7-AAD), propidium iodide (PI), and ethidium bromide (EtBr) can pass through the pyroptotic cells.

As opposed to pyroptotic cells, apoptotic cells preserve membrane integrity, making them immune to staining by these dyes. Annexin V staining pyroptotic cells is intriguing since it attaches to phosphatidyl serine (PS) and works similarly to apoptotic cells. As a result, pyroptotic cells and apoptotic cells cannot be distinguished by Annexin V. Furthermore, during apoptosis, apoptotic bodies are created, but during pyroptosis, pyroptotic bodies are formed. It's interesting to note that pyroptotic and apoptotic entities have comparable diameters and sizes, ranging from 1 to 5 μm. Additionally, there is a unique type of DNA damage that differs from apoptosis in that it manifests in the early stages of pyroptosis and is detected by positive dUTP nick-end labelling (TUNEL) staining.

In pyroptotic cells, DNA damage is less severe than in apoptotic cells. Whereas the apoptotic DNA fragment is organized and the nucleus is fragmented, the pyroptotic DNA fragmentation is random and the nucleus stays intact. It's interesting to note that pyroptosis and apoptosis are related to caspase activation. Pyroptosis was first thought to be a kind of cell death associated with caspase-1. Remarkably, recent research has demonstrated that other caspases, such as caspase-3/4/5/6/8/9/11, can induce pyroptosis in several cell types and are important players in both innate immunity and carcinogenesis. Although it was traditionally believed that caspase-3 carried out apoptosis, it has been proposed that caspase-3 can also cause pyroptosis by cleaving GSDME.

Surprisingly, pyroptosis can also be induced by directly cleaving GSDMD by the apoptosis-related enzyme caspase-8. Furthermore, via cleaving and activating caspase-3, caspase-9 activation also contributes to pyroptosis, while caspase-6 facilitates the cleavage of GSDMC. While caspase-2, caspase-7, and caspase-10 are only linked to apoptosis, current research indicates that caspase-1, caspase-4/5/11, and caspase-10 are only related to pyroptosis. However, as research continues, more links between caspases and both pyroptosis and apoptosis may be revealed one after the other. The amount of ATP present influences apoptosis, and ATP is depleted when apoptosis is coupled with PARP activation. Nonetheless, the gasdermin family comprises the pyroptosis effector proteins.

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Table 1 Similarities and differences between pyroptosis and apoptosis (Yu et al., 2021)

Characteristics	Pyroptosis	Apoptosis
Similarities		
Program cell death	+	+
PS exposure	+	+
Annexin V staining	+	+
TUNEL staining	+	+
DNA damage	+	+
Chromatin condensation	+	+
Membrane blebbing	+	+
Diameters of Pyroptotic and Apoptotic bodies	+	+
Caspase-3 activation	+	+
Caspase-6 activation	+	+
Caspase-8 activation	+	+
Caspase-9 activation	+	+
Differences		
Inflammation	+	-
Intact nucleus	+	-
Pore formation	+	-
Cell swelling	+	-
Cell shrink	-	+
Osmotic lysis	+	-
Membrane integrity	-	+
7-AAD staining	+	-
PI staining	+	-
EtBr staining	+	-
Caspase-1 activation	+	-
Caspase-4 activation	+	-
Caspase-5 activation	+	-
Caspase-11 activation	+	-
Caspase-2 activation	-	+
Caspase-7 activation	-	+
Caspase-10 activation	-	+
PARP cleavage	-	+
Gasdermin cleavage	+	-

PYROPTOSIS IN FISH

Pyroptosis is a form of programmed cell death that plays a crucial role in the host's defense against pathogenic infections. It is orchestrated by inflammasomes, which activate caspases and trigger the release of proinflammatory cytokines. Unlike mammals, fish have only one identified gasdermin (GSDME), which can be cleaved by caspase-a/-b to induce pyroptosis. Pyroptosis in fish primarily depends on the NLR family pyrin domain-containing 3 (NLRP3) inflammasome.

Specific Examples of Fish Pyroptosis (Xia et al., 2021; Song et al., 2022; Cao et al., 2023):

- **Zebrafish:** Bacterial hemolysin triggers pyroptosis in zebrafish during *Edwardsiella* infection.

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- **Japanese Flounder:** NLRP3 inflammasome activates caspase-1, leading to IL-1b maturation during *Edwardsiella piscicida* infection.
- ***E. tarda* Infection:** Caspase-1-mediated pyroptosis controls infection.
- ***Listeria monocytogenes* Infection:** Ectopic flagellin production (Lm-pyro) reduces virulence.
- ***Aeromonas veronii* Infection:** Neutrophils contribute to eradicating bacteria and inducing pyroptosis in goldfish.
- **Virus-Induced Pyroptosis:** Fish haemorrhagic virus induces pyroptosis, reducing macrophage counts.
- **Environmental Contaminants:** Toxicants and heavy metals can also induce pyroptosis in aquatic animals, impacting immune system function.

CONCLUSION

Current research on fish pyroptosis remains insufficient, with most studies primarily focusing on induction by harmful microorganism infections. However, it's essential to recognize that the aquatic environment in which fish reside is influenced by a variety of circumstances. NLRP3 inflammasomes play a pivotal role in fish pyroptosis. Future research should explore the involvement of other discovered inflammasomes, such as NLRCs, in fish pyroptosis. Additionally, pyroptosis holds promise as an effective technique for disease management in aquaculture. Targeting inflammasome activation and pyroptosis may mitigate the severity of infectious diseases while reducing economic losses. Furthermore, pyroptosis could contribute to the development of vaccines and immunostimulants to enhance fish immune responses.

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