JOURNAL OF LEUKOCYTE BIOLOGY

Journal of Leukocyte Biology, 2026, **118**, qiag008

https://doi.org/10.1093/jleuko/qiag008
Advance access publication 17 February 2026
Review

Neutrophils and the NLRP3 inflammasome: a tale of proteases, kinases, and inflammation

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Abstract

Neutrophils are key first responders to both infectious and noninfectious stress, playing a pivotal role in maintaining homeostasis through tightly regulated activation states and the release of inflammatory mediators. The Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is a key regulator of inflammation and has been extensively studied in monocytes and macrophages. However, recent research has shifted focus to the NLRP3 inflammasome in neutrophils and has highlighted its role in neutrophil activation and the production of inflammatory mediators. For example, neutrophils express a functional NLRP3 inflammasome with activation dynamics similar to those observed in monocytes. Canonical inflammasome activation is triggered by stimuli such as lipopolysaccharide and adenosine triphosphate via P2X7 receptor signaling, leading to interleukin-1β release. However, neutrophils also exhibit distinct characteristics, including the involvement of proteases other than caspase-1, differential regulation by kinases such as Bruton's tyrosine kinase, and the release of neutrophil extracellular traps and neutrophil proteases upon NLRP3. Moreover, apoptosis-associated speck-like protein containing a CARD (ASC)0–independent Nod-like receptor family pyrin domain containing 3 functions have been described. A picture emerges in which the interplay between NLRP3 activation and unique neutrophil functions is critical in various pathological contexts, yet the mechanisms and downstream effects remain underexplored. With a particular emphasis on the human system, this review aims to summarize current knowledge of NLRP3 inflammasome function in neutrophils. Given the essential need to consider the role of neutrophils in NLRP3–targeting approaches, it also seeks to highlight critical open questions that warrant further research.

Keywords: inflammasome, neutrophils, NLRP3

Key concepts

- Neutrophil-specific Nod-like receptor family pyrin domain containing 3 (NLRP3) regulation: neutrophils display unique inflammasome regulation, involving noncanonical pathways, alternative proteases, and posttranslational modifiers, such as Bruton's tyrosine kinase (BTK) and peptidyl arginine deiminase 4.
- Inflammasome-driven NETosis: NLRP3 activation in neutrophils promotes neutrophil extracellular trap (NET) formation, linking DNA, RNA, and protease release to inflammatory amplification and tissue damage.
- Protease-mediated interleukin-1β (IL-1β) processing: unlike macrophages, neutrophils can process IL-1β via serine and metalloproteases, revealing alternative inflammasome effector mechanisms.

Open questions

- What are the precise molecular mechanisms underlying BTK's dual regulatory role in NLRP3 activation in neutrophils?
- How do neutrophil-specific proteases like matrix metalloproteinase-9 and elastase influence IL-1β and gasdermin D processing independently of caspase-1?
- Is there a threshold of NLRP3 activation in neutrophils that shifts the balance from sublytic cytokine release to full pyroptosis or NET extrusion?
- Could neutrophil-targeted NLRP3 modulation reduce immunopathology without compromising host defense in viral or autoimmune diseases?

1. Introduction

There is a well-established link between inflammasome and a range of infectious and noninfectious inflammatory diseases, including cardiovascular, neurodegenerative, and metabolic disorders.[1] With the advances in research on inflammasomes over the past decades, it has become increasingly evident that they can play a protective or causal/contributing role in multiple disease onset and progression.[2]

As a response aimed at resolving a threat, inflammation is triggered by so-called pattern-recognition receptors (PRRs), which

Received: July 25, 2025. **Revised:** December 10, 2025. **Accepted:** December 29, 2025. **Corrected and Typeset:** February 17, 2026

sense threats from the outside via microbe-associated molecular patterns (MAMPs), but also from within, ie sterile settings, via danger-associated molecular patterns (DAMPs).[3] Multiple families exist among mammalian PRRs, with the toll-like receptors being the first to be identified. These sense extracellular MAMPs and DAMPs to initiate a first-level response by activating cytokine transcription. Sensors from the Nod-like receptor (NLR) family of PRRs, such as NLRP3, are cytoplasmic proteins that detect patterns indicating that the cytosol of a cell has been compromised by an invasive pathogen or damage. A more fulminant inflammation is thus initiated upon their activation, which is sometimes accompanied by cell death. Certain NLRs are notorious for engaging in multiprotein complexes, so called inflammasomes. These complexes are multiprotein molecular machineries that consist of a PRR, an adaptor termed "apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD)" or ASC, and an effector enzyme, such as caspase-1.[3,4] Caspase-1, in turn, cleaves the inactive pro-IL-1β and pro-IL-18 cytokines into mature and potent IL-1β and IL-18 to trigger full-scale inflammation and subsequent immune responses. However, also other active "alarmins" or DAMPs are released upon inflammasome activity without prior processing, eg high-mobility group protein B1 (HMGB1) or S100 proteins.[1] Additionally, caspase-1 may cleave gasdermin D (GSDMD), an initiator of inflammatory cell death known as pyroptosis.[1,2]

Until recently, inflammasome activation has mostly been studied in monocytes and macrophages. Here, a 2-step process, involving recognition and activation, is required to trigger inflammation (extensively reviewed in Weber et al.[1]). The priming step entails the transcription and production of key components of the inflammasome pathway, induced by MAMPs or DAMPs, eg via TLRs, and the first wave of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), IL-6, and IL-1β.[1,3] Moreover, there is posttranslational priming, involving the removal of inhibitory posttranslational modifications (PTMs) or the addition of activating modifications. The second step involves complex oligomerization to form a full inflammasome, including ASC and caspase-1, which, in the case of NLRP3, is triggered by a wide range of stimuli, including crystals, extracellular adenosine triphosphate (ATP), toxins, and a variety of pathogens,[1,3,4] that seem to converge on efflux of K^+ as a general signal that cellular integrity has been compromised. Apart from K^+ efflux, mitochondrial dysfunction, lysosomal damage, and cathepsin release have also been shown to trigger trans-Golgi network (TGN) dispersion and microtubule organizing center (MTOC) colocalization, which drive NLRP3 engagement of ASC and caspase-1 and thus, NLRP3 oligomerization; however, alternative pathways have also been described.[5–9] Furthermore, other players may be involved in the amplification of the activation step, such as the never in mitosis gene A-related kinase 7 (NEK7) activity. NEK7 acts downstream the K^+ efflux and directly interacts with NLRP3, thereby amplifying complex assembly.[10,11] These multiprotein assemblies are visible as so-called ASC specks.[12]

Following canonical NLRP3 activation, caspase-1 cleaves GSDMD, whose N-terminal fragments can form plasma membrane pores leading to pyroptosis.[13] Moreover, cytokines (including IL-1β and IL-18) and other cellular contents are released through these pores. The membrane protein Ninjurin-1 (NINJ1) has been implicated in pyroptosis, as well as in panoptosis and ferroptosis,[14] by promoting plasma membrane rupture (PMR) downstream of GSDMD cleavage. Upon activation, the extracellular α-helices of NINJ1 insert into the plasma membrane and polymerize into filamentous structures that destabilize lipid bilayers and lead to irreversible PMR.[15] Beyond its role in pyroptosis, NINJ1-dependent membrane damage facilitates the release of DAMPs, such as HMGB1, and can amplify inflammation, establishing a link between pyroptotic and secondary necrotic pathways.[15]

Figure 1 summarizes the general mechanisms of canonical NLRP3 inflammasome activation and the downstream events leading to pyroptosis as observed in monocytes or macrophages. In addition to the well-described NLRP3 canonical activation, alternative signaling pathways have been reported. A noncanonical inflammasome activation pathway has been identified, where intracellular lipopolysaccharide (LPS) is directly detected by caspase-4/5 in humans and caspase-11 in mice. This detection triggers caspase-1 oligomerization and autoproteolytic cleavage, leading to the activation of these caspases[16] independently of NLRP3. Once activated, the caspases cleave GSDMD, resulting in pore formation, the release of alarmins, and ionic imbalances, which may subsequently promote the activation of an NLRP3 inflammasome response.[16]

Another alternative pathway has been described in human monocytes following TLR4 activation, in which the aforementioned steps of priming and activation, which are hallmarks of the canonical pathway, occur in tandem.[5] TLR8-mediated NLRP3 activation has also been observed in macrophages or peripheral blood mononuclear cells (PBMCs), as well as in monocytes, in response to TLR8 ligands such as VTX-2337 or R848, leading to IL-1β and IL-18 release.[17] This alternative pathway is also activated during certain RNA viral infections, such as SARS-CoV or HIV.[18] However, the molecular mechanisms underlying this activation remain poorly understood. What is known is that this process involves K^+ efflux and other key players, such as CASP8 and RIPK1/3,[18–20] but does not typically lead to pyroptosis. Additionally, other triggers, such as neutrophil extracellular trap (NET)-associated RNA (naRNA) derived from neutrophil activation, may also induce this alternative pathway in both PBMCs and neutrophils.[21] Figure 2 summarizes the general mechanisms of noncanonical and alternative activation of the NLRP3 inflammasome.

Independent of the NLRP3 signaling pathway, traditional, inflammasome research has focused primarily on some myeloid subsets, especially monocytes and macrophages. However, in recent years, attention has shifted toward exploring the presence and role of the NLRP3 inflammasome in other leukocyte populations, including neutrophils. Here similarities but also striking differences—both in terms of signaling pathways as well as cellular outcomes—have emerged. Since any therapeutic approach that targets the NLRP3 inflammasome must prove effective in the most abundant leukocyte population, the neutrophils, the biology of neutrophil NLRP3 is essential to take note of.

The primary objectives of this review are therefore to (i) summarize recent findings on the unique features of NLRP3 inflammasome activation and regulation in neutrophils, (ii) highlight their potential links to various pathological conditions, and (iii) discuss translational implications.

2. Known triggers of NLRP3 inflammasome activation in neutrophils: mechanisms and implications

Past investigations into the dynamics of NLRP3 inflammasome activation in neutrophils have revealed multiple similarities and differences when compared with other well-known leukocytes.

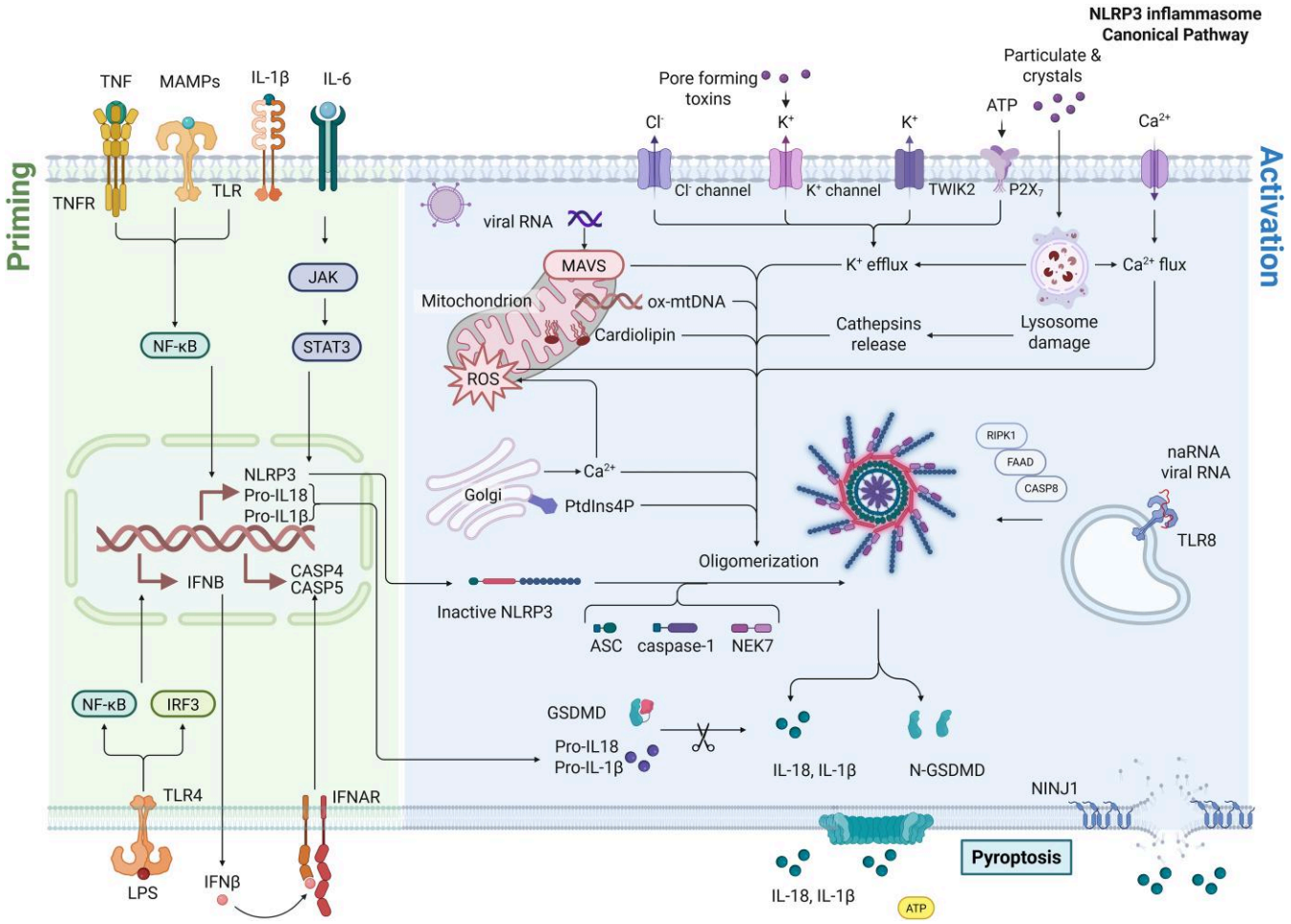

Fig. 1. Classical mechanisms of canonical NLRP3 inflammasome activation pathways. The activation of the NLRP3 inflammasome occurs through a 2-step process. The first step, known as priming, is triggered by engagement of pattern-recognition or cytokine receptors such as TNFR, TLRs, IL-1R, or IL-6R. This leads to NF-κB and STAT3 signaling, resulting in the transcriptional upregulation of NLRP3, pro-IL-1β, and pro-IL-18, as well as PTMs that prepare the NLRP3 complex for activation. The second step, referred to as Signal 2, involves direct activation of NLRP3 by a wide range of agonists. Many of these stimuli induce potassium efflux, including pore-forming toxins (eg nigericin), amyloid aggregates, ion channel modulators, or lysosomal destabilization. Other signals act independently of K^+ efflux, often through mitochondrial stress and the generation of mitochondrial-derived activators, such as oxidized mtDNA or cardiolipin. Upon activation, NLRP3 undergoes a conformational change that allows sequential recruitment of the adaptor ASC and pro-caspase-1, a process that may involve NEK7 as a stabilizing factor. Within the assembled inflammasome complex, caspase-1 cleaves pro-IL-1β, pro-IL-18, and GSDMD. Cleaved GSDMD forms membrane pores that facilitate cytokine release and induce pyroptotic cell death. Downstream of GSDMD pore formation, NINJ1 acts as an additional executor of PMR, amplifying the release of intracellular DAMPs and inflammatory mediators. Modified from *Nat Rev Immunol* (https://BioRender.com/ihtmvye) using BioRender.com.

Although neutrophils have classically been ascribed a limited transcriptional capacity—partly due to their short lifespan in circulation—a wide range of transcription factors may be involved in regulating gene expression during inflammatory and immune responses, including those related to the NLRP3 inflammasome. For example, neutrophils constitutively express nuclear factor kappa B (NF-κB) subunits, including RelA/p65, c-Rel, and p50, and exposure to stimuli such as LPS, TNF-α, or fMLP,[22] triggers the phosphorylation of IKK kinases and the degradation of the inhibitory IκB-α in the cytoplasm and nucleus. This leads to the activation and nuclear translocation of NF-κB, promoting the transcription of multiple inflammatory genes, such as cytokines (TNF-α, IL-1β, and IL-6) and chemokines (CXCL2, CXCL8, and CXCL10).[22–27] This chain of events can occur within the short lifespan of neutrophils and directly influences their inflammatory effector functions and multiple signaling pathways, including those associated with inflammasome activation.

When compared with macrophages or monocytes, neutrophils exhibit lower expression levels of NLRP3.[28,29] Moreover, although evidence for posttranslational priming in neutrophils is limited, this is likely, since canonical NLRP3 activation occurs in polymorphonuclear (PMN) leukocytes. For example, in both human and murine neutrophils, multiple MAMPs and DAMPs are known to prime and trigger NLRP3 activation, including LPS and ATP, which signal through the purinergic P2X7 receptor. Similar to what occurs in monocytes and macrophages, also in neutrophils ATP recognition by the purinergic receptor serves as a secondary signal for inflammasome assembly, inducing potassium efflux and increasing calcium (Ca^{2+}) influx. Additionally, IL-1β release was detected in response to other NLRP3-related stimuli, such as the pore-forming toxin nigericin in combination with LPS. Interestingly, in these studies, no significant induction of neutrophil pyroptosis was observed upon NLRP3 activation, contrasting with the pronounced pyroptotic response of human and murine macrophages.[29–31]

NLRP3 activation was also shown in several in vitro infection models of human and murine neutrophils, eg with *Pseudomonas aeruginosa*,[32] *Staphylococcus aureus*,[33] and *Streptococcus*

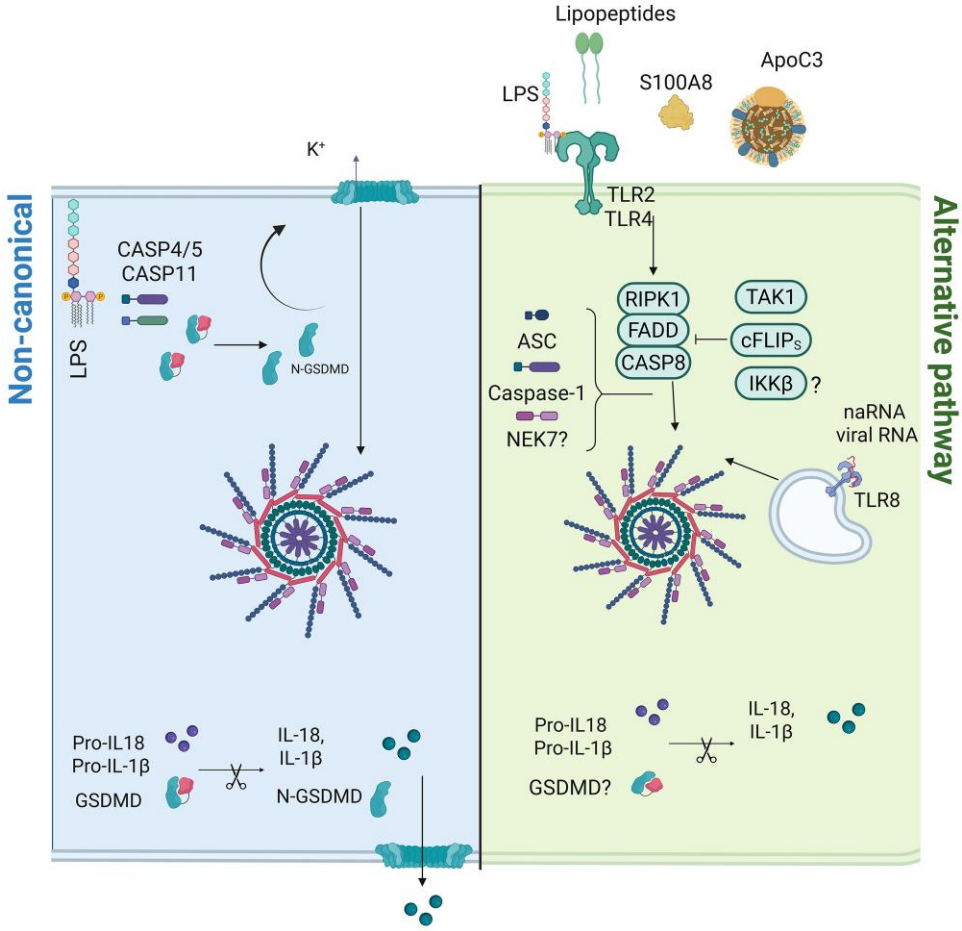

Fig. 2. Noncanonical and alternative pathways of NLRP3 inflammasome activation. Noncanonical inflammasome activation involves intracellular recognition of LPS by caspase-4 and caspase-5 in humans (or caspase-11 in mice). Once activated, these caspases cleave GSDMD, whose N-terminal fragment forms pores in the plasma membrane. Pore formation promotes potassium (K^+) efflux, which can secondarily trigger NLRP3 inflammasome assembly and activation. In contrast, alternative NLRP3 activation pathways are characterized by a 1-step mechanism in which cell-surface receptors, such as TLR2 and TLR4, directly initiate inflammasome activation without requiring a distinct priming phase. In monocytes, this results in IL-1β release without pyroptotic cell death. These NETs may contain NLRP3-stimulatory ligands, including naRNA and the antimicrobial peptide LL-37. DAMPs, danger-associated molecular patterns; MAMPs, microbe-associated molecular patterns; PRR, pattern-recognition receptor; ROS, reactive oxygen species; mtDNA, mitochondrial DNA. Modified from *Nat Rev Immunol* (https://BioRender.com/ihtmvye) and created using BioRender.com.

pneumoniae.[29,30,34] Interestingly, these investigations suggest that, depending on the neutrophil source used (eg peripheral blood, tissue, bone marrow, murine, or human), the priming step during bacterial infection may be mediated by different PRRs, including TLR2, TLR4, and NOD2.[29,30,34]

Noncanonical inflammasome activation has also been described in neutrophils. Studies have shown that transfection of neutrophils with LPS[35,36] activates caspase-11 and caspase-1 similarly to macrophages, leading not only to IL-1β release and pyroptosis, but also to NET extrusion. Interestingly, the same pattern of caspases activation and cytokine/NET release was observed in an *Aspergillus fumigatus* infection model.[37]

naRNA has also emerged as a trigger of noncanonical NLRP3 activation in neutrophils. Bork et al. showed that naRNA, together with the antimicrobial peptide LL37, can engage naive neutrophils via a mechanism that is still uncertain and seems to involve recognition by human TLR8 (or Tlr13 in mice). This process leads to NLRP3 activation and establishes a feed-forward loop of activation and NET formation.[21,38–40] Furthermore, MAMPs and DAMPs are not the only group of molecules capable of affecting NLRP3 activation/priming in neutrophils. It appears that growth factors such as GM-CSF induce priming through JAK/STAT signaling, as well as IL-1 release via a mechanism that remains unclear.[41]

Another study demonstrated that E-selectin engagement triggers Bruton's tyrosine kinase (BTK)-dependent NLRP3 phosphorylation, potassium efflux, and GSDMD-dependent pore formation, ultimately leading to the cytosolic release of DAMPs such as S100A8/S100A9.[42]

Furthermore, a notable distinction between neutrophils and macrophages with respect to NLRP3 activation was reported in the context of *Yersinia* or *Salmonella* infection. In macrophages, the type 3 secretion system serves as a key MAMP to trigger leukotriene B4 (LTB4) synthesis in an NLRP3-dependent manner. However, this pathway does not seem to be active in neutrophils. Instead, LTB4 synthesis in neutrophils depends on the SKAP2/PLC pathways, highlighting intrinsic differences between cell types.[43] Conversely, neutrophil recruitment to sites of inflammation in response to high concentrations of LTB4 appears to be strongly dependent on NLRP3 inflammasome activation.[44] Table 1 summarizes different infection models as well as MAMPs and sterile molecules known to affect priming and/or activation of the NLRP3 inflammasome in both human and murine neutrophils.

3. Neutrophil-specific intracellular regulation

Considering that NLRP3 is activated by a broad variety of MAMPs and DAMPs under multiple conditions (Table 1), both infectious and sterile, this process must be tightly regulated to ensure an appropriate response to stress, generate effective pathogen defense, maintain self-tolerance, and prevent the development of autoimmunity or chronic inflammatory diseases.

One of the key regulatory mechanisms is PTM, which has been extensively explored in monocytes and macrophages. These modifications include ubiquitination and deubiquitination of NLRP3, phosphorylation and dephosphorylation of multiple components of the NLRP3 pathway, acetylation/deacetylation, SUMOylation, nitrosylation, citrullination, and more.[70] These processes are mediated by various partners and have different consequences for NLRP3 assembly. Here, we summarize some of these modifications, highlighting unique features of NLRP3 regulation in neutrophils compared with other leukocytes.

First, a classic, well-known NLRP3 regulator is the kinase BTK. In macrophages, BTK has been shown to play a critical role in enhancing NLRP3 function, thus promoting optimal IL-1β release. As a result, patients with a genetic immunodeficiency known as X-linked agammaglobulinemia (XLA) and individuals treated with U.S. Food and Drug Administration-approved BTK inhibitors, such as ibrutinib, exhibit reduced IL-1β release ex vivo.[71]

Multiple experimental models of NLRP3 activation have demonstrated a positive regulatory role for BTK.[71–74] At the molecular level, BTK was found to interact constitutively with NLRP3 in macrophages, driving TGN dissociation and facilitating NLRP3 oligomerization through direct phosphorylation of 3 adjacent tyrosine residues on NLRP3, enhancing NLRP3 activity.[73] However, conflicting results were obtained in a study by Mao et al.[75] suggesting that at higher LPS concentrations, BTK may inhibit NLRP3 activity. These findings highlight the dual role of BTK in regulating NLRP3 activity.

A recent study explored for the first time the dual function of BTK in both neutrophils and macrophages.[60] The study revealed that genetic BTK ablation or treatment with BTK inhibitors resulted in increased NLRP3 activity and IL-1β release in primary murine and human neutrophils, contrasting with a reduction in matched bone marrow–derived macrophages or PBMCs from Btk-deficient or XLA patients. Suppression of BTK activity also increased NET formation, suggesting that BTK acts as a novel negative regulator of NLRP3 in neutrophils.[60] The observed effects were also independent of the LPS priming concentration, as suggested by Mao et al.,[75] emphasizing that BTK may regulate NLRP3 inflammasome activation in a different, as-yet unexplored way, depending on the cell type.

The exact molecular mechanisms behind the negative regulation of NLRP3 by BTK in neutrophils remain unclear, but 1 hypothesis is that BTK may modulate phosphatase PP2A activity, potentially preventing NLRP3 activation by inhibiting dephosphorylation at specific sites, as suggested by Mao et al.[75] Alternatively, BTK may prevent excessive NLRP3 activation through a mechanism involving reactive oxygen species (ROS) production,[76] although this link has been debated.[77]

In parallel, inflammasome activation may indirectly modulate the availability and activity of the matrix metalloproteinase (MMP)-9, which in turn contributes to IL-1β processing and NET formation in neutrophils. As previously described, NLRP3 activation can facilitate cytosolic release of granular proteases through GSDMD pore formation, potentially enhancing MMP-9-mediated cleavage of pro-IL-1β. In our recent study,[60] inhibition of NLRP3 with MCC-950 reduced IL-1β and MMP-9 levels, while selective inhibition of MMP-9 significantly decreased IL-1β production and NET release. These findings indicate that MMP-9 acts downstream of NLRP3, participating in IL-1β maturation and NET extrusion. However, the precise mechanisms—such as potential dependence on GSDMD or whether MMP-9 can directly process GSDMD—remain to be elucidated. Interestingly, IL-1β has been reported to transcriptionally upregulate MMP-9 expression in other myeloid and nonmyeloid cells.[78,79] This suggests that a similar feedback mechanism may also operate in neutrophils, where inflammasome activation could enhance MMP-9 activity both by increasing its cytosolic availability and by inducing its expression, thereby amplifying inflammatory and NET-related responses.

Another key player in NLRP3 regulation in neutrophils is the peptidyl arginine deiminase 4 (PAD4). Münzer et al.[57] described PAD4 as an important positive regulator acting on NLRP3 and ASC in the cytoplasm. This effect may be explained by the fact that the conversion of arginine to citrulline alters the charge of the protein, directly influencing, protein–protein interactions and subsequent assembly of the NLRP3 inflammasome, an event that has also been observed in macrophages.[80] Importantly, recent work has shown that PAD4 may also citrullinate NF-κB p65, enhancing its nuclear localization and transcriptional induction of inflammasome-related cytokines,[81] which further supports a mechanism whereby PAD4 modulates priming signals in neutrophils. The unique feature of PAD4 in neutrophils is its ability not only to act directly on inflammasome components but also to participate in the formation and extrusion of NETs.[57]

Poly(ADP-ribose) polymerase-1 (PARP-1), a key enzyme in DNA repair, is also recognized as a regulator of NLRP3. According to Chiu et al.,[82] in macrophages, PARP-1 translocate to the cytosol upon ATP stimulation, initiating PARylation of NLRP3, increasing ROS levels and influencing interaction of NLRP3 with other partners like TXNIP, ultimately promoting inflammasome activation. In neutrophils, it has recently been reported that PARP-1 regulates NLRP3 levels, ASC dimerization, and NLRP3-dependent NET formation, independent of pyroptosis.[69] Mechanistically, PARP-1 appears to act as a negative regulator of the MAPK p38, which typically downregulates NLRP3 and NET formation,[69] though this mechanism requires further investigation.

Although multiple questions still remain open, recent advances in the understanding of neutrophil biology—especially NLRP3-related activation mechanisms and their influence on neutrophil function—have been achieved, including the role of kinases and other posttranslational regulators of inflammasome pathways.

4. Typical inflammasome outcomes with unique regulation in PMN

The relevance of IL-1β release and its relationship to cell death in neutrophils remain uncertain, with conflicting findings that have yet to be fully clarified. Most of the stimuli listed in Table 1 have been associated with IL-1β release, as typically observed in macrophages and monocytes. However, in neutrophils, IL-1β production is consistently much lower than in other leukocytes,[28,29] as evidenced by the studies summarized in Table 1. This phenomenon may be explained, at least in part, by the lower expression of NLRP3 in neutrophils compared with other cell types.[30,31]

Nevertheless, considering the abundance of neutrophils and their rapid response during inflammation, even low levels of IL-1β production likely retain physiological relevance. This

Table 1. Previously described NLRP3 inflammasome activators in neutrophils.

Agonist	Redout(s) for NLRP3 priming/activation in PMNs	Cellular source	Other outcomes related to NLRP3	Reference
Priming effect: human PMNs				
Infection model				
Ivt—H. pylori 3h-24h	NLRP3 and ASC (W)	huPMNs (HD)	…	45
Ivt—E. coli 3h	NLRP3 (W)	huPMNs (HD)	…	46
MAMPs/sterile molecules				
LPS (10 ng/mL) 4 h	*NLRP3*, *IL1B* (PCR)	huPMNs (HD)	…	47
Serum amyloid A (5 µg/mL) 2 to 8 h	NLRP3 and pro-IL-1β (W) *NLRP3* and *IL1B* (PCR)	huPMNs (HD)	…	48
TNF-α (10 to 50 ng/mL) 24 h	*IL1B* (PCR) NLRP3 (W)	huPMNs (HD)	…	49
Serum Amyloid A (10 µg/mL) 24 h	NLRP3 (W)	huPMNs (HD)	…	50
Priming effect: murine PMNs				
Infection model				
Iv—Str. pneumoniae	NLRP3 and ASC (W)	Peripheral blood msPMNs (C57BL/6)	…	30
Iv—Str. pneumoniae	Pro-IL-1β (F) NLRP3 (W)	Pulmonary msPMNs (C57BL/6)	…	34
Iv—H. pylori	Pro-IL-1β (Wb) *NLRP3* and *IL1B* (PCR)	Peritoneal msPMNs and msBMNs (C57BL/6)	…	51
Staphylococcal enterotoxin O (1 µg/mL) 4 to 8 h	*NLRP3* (PCR)	Peritoneal msBMNs (C57BL/6)	…	52
MAMPs/sterile molecules				
LPS (200 ng/mL) 4 h	*NLRP3* (PCR)	msBMNs (C57BL/6)	…	53
PMA (100 nM) 3 h	*NLRP3*, *CASP1*, *GSDMD* (PCR)	msBMNs (DBA/1)	…	54
Activation effect: human PMNs				
Infection model				
Ivt—heat-killed *Str. pneumoniae* 3 h + *Str. pneumoniae* TIGR4 or highly purified active hemolytic pneumolysin	IL-1β (E) Active caspase-1 (F)	huPMNs (HD)	hCXCL8 (E) K + Efflux (Lu)	30
Ivt—H. pylori 3 to 24 h	IL-1β (E) Cell death (LU) ASC "specks" (IF) Active caspase-1 (F)	huPMNs (HD)	…	45
Ivt—E. coli 3 h	IL-1β (E) Caspase-1 cleavage (W) Cell death (C)	huPMNs (HD)	ROS (Lu) Phagocytosis (F)	46
MAMPs/sterile molecules				
LPS (200 ng/mL) 4 h + Nigericin (6.5 µM) 3 h	IL-1β (E)	huPMNs (HD)	…	53
LPS (10 ng/mL) 4 h + ATP (5 mM) or Nigericin (5 µM) 2 h	IL-1β (E)	huPMNs (HD)	…	47
LPS (500 ng/mL) 4 h + ATP (4 mM) 45 min or Nigericin (10 µM) 45 min	IL-1β (E) IL-1β and caspase-1 cleavage (W)	huPMNs (HD)	Cytosolic calcium increase (LU) K + Efflux (LU)	29
TNF-α (500 ng/mL) 3 h + bacterial-derived toxin pneumolysin (Ply; 500 ng/mL) 1.5 h	IL-1β (E)	huPMNs (HD)	…	34
LPS (1 µg/mL) 4 h + ATP (5 mM) 1 h	IL-1β (E) IL-1β cleavage (W)	huPMNs (HD)	S100A8 (E) TNF-α (E)	55
PMA (100 nM) 0.5 to 4 h	Cell death (IF) GSDMD pore formation (IF) GSDMD cleavage (W)	huPMNs (HD)	ROS (F) Phagocytosis (IF) NET (IF)	56
TNF-α (50 ng/mL) 12 h + MSU (200 µg/mL) 12 to 24 h	IL-1β (E) Caspase-1 (p20) (E) IL-18 (E)	huPMNs (HD)	…	49
Nigericin (15 µM) 4 h or Ionomycin (4 µM) 4 h	ASC specks (IF) Caspase-1 cleavage (W)	huPMNs (HD)	NET (IF)	57
LPS (5 µg/mL) 0.5 to 4 h	ASC "specks" (IF) IL-1β (E)	huPMNs (HD)	NET (IF)	58
LPS (1 µg/mL) 3 h + ATP (3 mM) 45 min or + sUA (100 µM) 45 min or β-glucan (200 µg/mL) 3 h + ATP (3 mM) 45 min	IL-1β (E) Active caspase-1 (F) NLRP3 + ASC + "specks" (IF)	huPMNs (HD; COVID)	NET (IF/Pi)	59
LPS (10 to 100 ng/mL) 3 h + ATP (5 mM) 1 h or + Nigericin (5 µM) 2 h	IL-1β (E) Active caspase-1 (F) NLRP3 + ASC + "specks" (IF)	huPMNs (HD)	NET (IF/Pi) MMP-9 expression (IF) MMP-9 release (E)	60

(*continued*)

Table 1. Continued

Agonist	Redout(s) for NLRP3 priming/activation in PMNs	Cellular source	Other outcomes related to NLRP3	Reference
NET-associated RNA (naRNA) + LL37	IL-1β (E)	huPMNs (HD)	NET (IF)	21
Activation effect: murine PMNSs				
Infection model				
Iv/Ivt—Str. pneumoniae	IL-1β (E) Pro-IL-1β (F) Caspase-1 and Pro-IL-1β cleavage (W) Active caspase-1 (F/IF) NLRP3 oligomers (IF) ASC "specks" (IF) ASC oligomerization (W)	msBMNs and Corneal msPMNs (C57BL/6)	…	30
Iv—Group B *Streptococcus*	IL-1β (F)	Intraperitoneal msPMNs (C57BL/6)	…	61
Ivt—Group B *Streptococcus* 2 to 24 h or LPS (100 ng/mL) 2 to 24 h + ATP (5 mM) 1 h	IL-1β (E) IL-1β cleavage (W)	BMNs (C57BL/6)	TNF-α (E)	61
Iv—Str. pneumoniae	IL-1β (E) IL-1β cleavage (W)	Pulmonary msPMNs (C57BL/6)	TNF-α (E)	34
Ivt—LPS (100 ng/mL) 4 h + *Salmonella typhimurium* or + Nigericin (5 μM) 1 to 5 h or Pam3CSK4 (1 mg/mL) transfection 4 h	Caspase-1 and GSDMD cleavage (W) IL-1β (E) Cell Death (IF)	msBMNs (C57BL/6)	NET (IF)	36
Ivt—H. pylori 24 h	IL-1β and caspase-1 cleavage (W) IL-1β (E)	Peritoneal msPMNs and msBMNs (C57BL/6)	…	51
MAMPs/sterile molecules				
DENV rEII (0.6 μM) 1 h	IL-1β (E) Cell death (F)	msBMNs (C57BL/6)	NET (F, IF) ROS (F)	62
Staphylococcal enterotoxin O (1 μg/mL) 3 h + 2 mM ATP 9 h	IL-1β (E) Pro-IL-1β, caspase-1, GSDMD cleavage (W)	Peritoneal msPMNs and msBMNs (C57BL/6)	TNF-α and IL-6 (E)	52
LPS (1 μg/mL) 4 h + ATP (5 mM) 1 h or + Silica (n.i.)	IL-1β release (E) IL-1β cleavage (W)	msBMNs (C57BL/6)	TNF-α (E) Cathepsin G and elastase activity (LU)	55
LPS (200 ng/mL) 4 h + Nigericin (6.5 μM) 3 h	IL-1β release (E) IL-1β cleavage (W)	msBMNs (C57BL/6)	…	53
LPS (500 ng/mL) 4 h + ATP (1 to 3 mM) 45 min	IL-1β release (E) Pro-IL-1β and caspase-1 cleavage (W)	msBMNs (C57BL/6)	Cytosolic calcium increase (LU)	29
LPS (100 ng/mL) 4 h + Nigericin (1.25 to 5 μM) 1 to 5 h	IL-1β release (E) Pro-IL-1β and caspase-1 cleavage (W)	msBMNs (C57BL/6)	…	63
NETs 12 h	NLRP3, Pro-IL-1β and casp-1 p20 (W) IL-1β cleavage (W) NLRP3 + ASC + "specks" (IF)	Rat PMNs from abdominal fluid (Sprague Dawley)	Tissue neutrophil infiltration (HQ)	64
LPS (0.25 μg/mL) 2.5 h + ATP (2.5 mM) 1 h or Nigericin (5 μM) 1 h	IL-1β (E) Pro-caspase-1 and Pro-IL-1β cleavage (Wb) Cell death (C) NLRP3 oligomers (IF) GSDMD cleavage (W)	msBMNs (C57BL/6)	ROS (FC)	65
Nigericin (15 μM) 4 h or Ionomycin (4 μM) 4 h	ASC "Specks" (IF) Caspase-1 cleavage (W)	msBMNs (C57BL/6)	NET (IF)	57
LPS (250 ng/mL) 2 h Nigericin (6.7 μM) 1 to 2 h	IL-1β (E) Pro-caspase-1 and Pro-IL-1β cleavage (W)	msBMNs (C57BL/6)	Spleen and liver damage (ICH)	66
Iv—Thioglycollate-induced peritonitis	ASC + Ly6G + (IF)	Peritoneal msPMNs (C57BL/6)		67
LTB4 (40 to 4,000 pg/mL) 1 h	ASC "specks" (IF)	Peripheral blood msPMNs (C57BL/6)	Chemotaxis (IF) MTOC dispersion (IF) Cell elongation (IF)	44
Ionomycine (4 μM) or β-cyclodextrin-pristane (26.6 μM) 1 h	ASC "specks" (IF)	Peripheral blood msPMNs C57BL/6)	NET (IF) Diffuse alveolar hemorrhage (IHC)	68
LPS (1 μg/mL) 3 h + Nigericin (15 μM) 45 min	NLRP3 (W)		NET (IF)	69

(*continued*)

Table 1. Continued

Agonist	Redout(s) for NLRP3 priming/activation in PMNs	Cellular source	Other outcomes related to NLRP3	Reference
	IL-1β (E) Pro-IL-1β (F) Caspase-1 and GSDMD cleavage (W)	Peritoneal msPMNs (C57BL/6)		
LPS (10 to 100 ng/mL) 3 h + ATP (5 mM) 1 h or + Nigericin (5 μM) 2 h	IL-1β (E) Active caspase-1 activity (F)	msBMNs (C57BL/6)	NET (IF/Pi) MMP-9 expression (IF) MMP-9 release (E)	60

C, colorimetric assay; E, ELISA; F, flow cytometry; HD, healthy donors; IF, immunofluorescence; IHC, immunohistochemistry; Iv, in vivo; Ivt, in vitro; LU, luminescent assay; msBMNs, mouse bone marrow neutrophils; n.i., not indicated; Pi, Picogreen; PMNs, polymorphonuclear cells; W, western blot.

process may contribute to the amplification and persistence of inflammation regardless of the nature of the trigger—infectious or sterile.[65]

An additional, distinctive aspect concerns the cleavage and release of pro-IL-18, another classic, inflammasome-dependent cytokine, whose regulation in neutrophils remains poorly defined. Only 1 study has reported IL-18 production by neutrophils: Bakele et al.[47] suggested that, while IL-1β production is strongly dependent on inflammasome activation, IL-18 may be generated independently of inflammasomes, contrasting with observations in other myeloid cells. However, several other studies have failed to detect IL-18 production in primary neutrophils following NLRP3 inflammasome activation.[29,30,34,60]

In terms of cell death, conflicting studies over the past years have questioned the sensitivity of neutrophils to pyroptosis, regarded as the canonical, NLRP3-dependent type of cell death in monocytes/macrophages. These findings have spurred research on the role of molecules such as GSDMD in neutrophils, with earlier investigations suggesting an apparent resistance of this cell type to cell death following inflammasome activation.[29,31,36,83] Nevertheless, noncanonical activation has been shown to trigger GSDMD-dependent cell death and NET extrusion in neutrophils.[36]

A study conducted by Chauhan et al.[66] using a gain-of-function NLRP3A350V mouse model suggested that canonical inflammasome activation induces GSDMD cleavage, IL-1β release, and pyroptosis. However, the authors found that GSDMD is dispensable for PMA-induced NETosis, implying that the trigger plays a critical role in determining the fate of the cell.

On the other hand, some authors posited that the low expression of NLRP3 components in neutrophils maintains these leukocytes in a sublytic state, enabling the release of IL-1β and NETs without inducing cell death.[84] This hypothesis is further supported by the proposition that effector enzymes, such as caspase-1, cleave pro-IL-1β more efficiently than GSDMD.[36]

Another factor that may influence neutrophil sensitivity to pyroptosis is the toll/interleukin-1 receptor motif-containing protein 1 (SARM1). While SARM1 has been identified as a key regulator of pyroptosis in macrophages,[85] it is expressed at significantly lower levels in neutrophils.[65]

Various forms of plasma membrane damage—including that caused by GSDMD pore formation—can trigger Ca^{2+} influx, which in turn activates the ESCRT-mediated membrane repair machinery. This process promotes the shedding of damaged plasma membrane fragments as extracellular vesicles.[86] Importantly, the ESCRT system mitigates cell lysis by delaying or modulating PMR and DAMP release, but does not interfere with GSDMD activation.[86] This suggests a finely tuned balance between ESCRT-dependent repair and NINJ1-mediated membrane rupture that could determine the timing and extent of pyroptotic progression.[87] However, how this balance is regulated in neutrophils and whether it influences their susceptibility to lytic cell death remain unclear.

Regarding the role of NINJ1 as an additional executioner of lytic cell death, recent studies indicate that NINJ1 oligomerization mediates PMR downstream of GSDMD during inflammasome-driven pyroptosis, thereby promoting the release of DAMPs such as HMGB1.[88] In a mouse model of acute oxalate nephropathy, myeloid NINJ1 drove macrophage PMR and HMGB1 release, which subsequently enhanced neutrophil activation and NET formation. Genetic or antibody-mediated inhibition of NINJ1 reduced DAMP release, NET accumulation, and tissue injury.[88]

Although these findings support a macrophage–neutrophil inflammatory axis mediated by NINJ1, other studies have suggested that NINJ1 is dispensable for GSDMD pore formation and GSDMD-dependent IL-1β release.[87,89] These conflicting results indicate that NINJ1 acts downstream of GSDMD, executing PMR rather than being required for pore formation or cytokine secretion. Thus, while NINJ1-dependent PMR amplifies inflammation through DAMP liberation, its neutrophil-autonomous role in NET extrusion or neutrophil death remains uncertain and requires further experimental clarification.

5. Beyond IL-1 and pyroptosis: neutrophil-specific NLRP3 inflammasome features

Although IL-1β is widely regarded as a primary product of inflammasome assembly, the consequences of NLRP3 activation in neutrophils extend far beyond cytokine release, significantly impacting their cellular biology and functionality. One of the unique effector mechanisms attributed to neutrophils is the formation of NETs.

NETs are web-like structures extruded by neutrophils, mainly composed of genomic DNA and a diverse range of proteins.[90] Multiple molecules have been described as associated with the NET structure, such as histones, HMGB1, cathelicidin LL37, and other neutrophil proteases, including myeloperoxidase (MPO), MMP-9, and elastase.[91] Moreover, more recently, naRNA has also been shown to be released during NET formation, along with DNA and proteins, and to contribute to their antimicrobial function and amplification of inflammation.[21]

Sollberger et al.[56] showed that GSDMD is activated in neutrophils during the generation of NETs and plays a vital role in NET release. Additionally, they observed that PMA-induced NETosis

was dependent on GSDMD cleavage, which occurred in a caspase-independent manner. Instead, the neutrophil elastase (NE) was shown to be the main protease responsible for GSDMD processing and NET release. Sollberger and colleagues further suggested that GSDMD forms pores in the granules, leading to the release of NE into the cytosol, thus amplifying GSDMD processing and NE activity. NE may also translocate to the nucleus, processing histones and expanding the nucleus, which could directly impact the extent of NETosis. This observation aligns with another study showing GSDMD processing by NE and a longer lifespan of neutrophils in the absence of GSDMD.[92]

The same dependence on GSDMD for NET release was also pointed by Chen et al., who established a direct link between noncanonical inflammasome activation and NET release.[36] Münzer et al.[57] were among the first to explore the relationship between NLRP3 activation and NET release using both in vitro and in vivo models. Their work showed that NET formation is directly regulated by NLRP3 inflammasome activation, not only in response to MAMPs but also under sterile conditions. The process also involves PAD4, which plays a dual role: it directly facilitates NET formation through histone citrullination and chromatin decondensation in the nucleus, while also contributing to posttranscriptional regulation of NLRP3 and ASC in the cytoplasm, enhancing inflammasome assembly.[57] Moreover, the study identified NLRP3 as essential for NET release by mediating nuclear and PMR. Subsequent studies reinforced the direct link between NLRP3 activation and NET formation in response to multiple DAMPs and MAMPs and during infectious diseases[58,60,62,68] (Table 1).

Another distinctive feature of neutrophil biology is the presence of alternative pathways for IL-1β maturation that operate independently of canonical inflammasome activation. Several neutrophil-derived proteases, including serine proteases such as elastase[32,93] and proteinase 3,[94–96] as well as metalloproteinases such as MMP-2, MMP-3, and MMP-9,[60,97] can directly cleave pro-IL-1β in the absence of caspase-1 activity. Additionally, granzyme A has also been implicated in IL-1β processing through inflammasome-independent mechanisms.[98] Although these proteases do not require inflammasome assembly, inflammasome activation may indirectly modulate their activity. For instance, GSDMD pore formation could promote the release of granular proteases into the cytosol, facilitating pro-IL-1β processing.

Consistently, studies have shown that the N-terminal fragment of GSDMD, generated during NLRP3 inflammasome activation, preferentially localizes to the membranes of azurophilic granules or autophagosomes, rather than the plasma membrane, which could also affect the extent of cell death. This localization influences the leakage of proteases such as elastase into the cytosol.[31] These findings also suggest that IL-1β release in neutrophils may be mechanistically dependent on autophagy.[31]

Moreover, IL-1β secretion itself may enhance the expression or activation of certain proteases, establishing a potential feed-forward loop that amplifies inflammatory signaling in neutrophils.[1] Further studies are needed to clarify these interconnections and their relevance to neutrophil-specific inflammasome dynamics. A recent study also indicates that lysosome disruption is capable of inducing IL-1β processing and release in an NLRP3-independent manner.[99] This effect may be ascribed to the activity of serine proteases, which, once stored in lysosome-like azurophilic granules, could directly cleave pro-IL-1β and GSDMD. This suggests a novel, neutrophil-specific signaling mechanism for lysosomal disruption-induced processing and export of IL-1β.[99]

Figure 3 summarizes the general mechanisms of NLRP3 inflammasome regulation and activation in neutrophils.

6. Inflammasome-dependent neutrophil-mediated immunological diseases

6.1 NET-related inflammatory and autoimmune diseases

Once IL-1β, NETs, proteases, or other DAMPs are released, the consequences of NLRP3 activation can be beneficial, contributing to infection control through direct effects or by modulating other innate or adaptive immune system players. However, they can also be detrimental, exacerbating the inflammatory response, leading to cytokine storms, tissue damage, and contributing to the pathology of various diseases. Excessive inflammation may result from an unbalanced release of IL-1β or, to a greater extent, from the abundant proinflammatory stimuli present in NETs, including a variety of proteases, the DNA by itself, and NET-associated RNA (na-RNA).[21]

Neutrophil proteases also contribute to the amplification of inflammation through mechanisms that indirectly intersect with inflammasome activation. For instance, MPO can engage CD11b to activate NF-κB and promote cytokine release, while NE enhances IL-8 production via the MyD88/IRAK/TRAF6 pathway, further sustaining neutrophil recruitment and activation.[100–106] In addition, the serine protease PR3 generates the antimicrobial peptide LL-37, which forms complexes with extracellular nucleic acids and activates Toll-like receptors, establishing a proinflammatory loop that may potentiate NLRP3 activation in subsequent responses.

Previous studies by Herster et al. (2020)[107] and Bork et al. (2024)[21] showed that besides neutrophils, also keratinocytes seem to be responsive to naRNA via NOD2-RIPK signaling, leading to the expression of psoriasis-related genes (eg IL-17 and IL-36) and thus likely contributing to inflammatory skin diseases.[21]

Another NLRP3-associated product, IL-18, may induce NET release in human neutrophils, although many studies have failed to show release of IL-18 by neutrophils.[108] NET release in turn leads to NLRP3 inflammasome activation in macrophages with IL-1β and IL-18 release, thus amplifying the activation state of neutrophils and contributing to the exacerbation of pathological conditions such as systemic lupus erythematosus (SLE).[108–110] In fact, one of the complications of SLE is diffuse alveolar hemorrhage (DAH), which has been previously associated with NLRP3-dependent NET formation.[111] Interestingly, in a mouse model of pristane-induced DAH, inflammasome activation and NET formation were also directly influenced by sex, being more pronounced in female animals.[68,111]

NET release has also been associated with a loop of NLRP3 activation in neutrophils and other leukocytes in multiple inflammatory and/or autoimmune diseases, including diabetic models,[64] a mice model for LPS-induced brain inflammation,[112] rheumatoid arthritis [54] and the experimental autoimmune encephalomyelitis model.[113,114] In the latter, a significant neutrophil infiltration in the brain and spinal cord of the animals was observed, alongside NLRP3-dependent NET formation, which was marked by increased CXCR2 and CXCR4 expression and dependent on ROS generation.

One of the hallmark features of inflammasome hyperactivation is endothelial damage, which is commonly observed in various inflammatory conditions. NLRP3 has been identified as a central player in endothelial activation through IL-1β release. Using a mouse model of thioglycolate-induced peritonitis, which is characterized by neutrophil influx, Fukui et al.[67] observed that

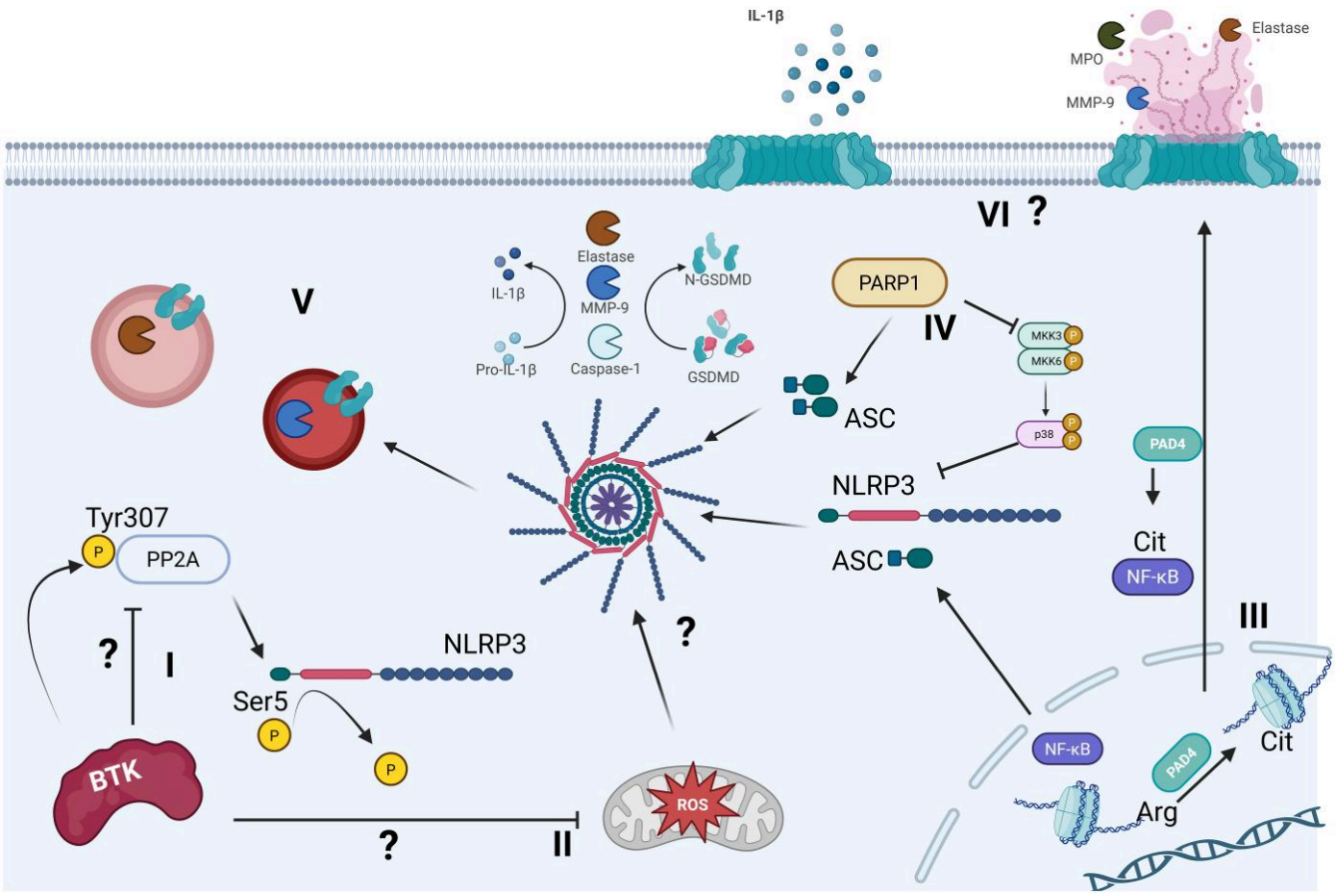

Fig. 3. Proposed mechanisms of NLRP3 inflammasome regulation and activation in neutrophils. Multiple pathways regulate the activation of the NLRP3 inflammasome in neutrophils: (I) BTK binds to PP2A and induces the phosphorylation of Tyr307, thus inhibiting PP2A-mediated dephosphorylation of Ser5 in the pyrin domain of NLRP3, and reducing NLRP3 inflammasome activation. (II) Release of ROS, typically produced by mitochondrial stress or NADPH oxidase, is controlled by BTK. Their role in NLRP3 activation is controversial (indicated by "?"). (III) PAD4 catalyzes histone citrullination in the nucleus, promoting chromatin decondensation and NET formation. In the cytoplasm, PAD4 also enhances NLRP3 inflammasome components by citrullinating NF-κB, thereby increasing its activity. (IV) PARP-1 negatively regulates p38 MAPK signaling, thereby restraining NLRP3 inflammasome activation, and also promotes ASC dimerization, contributing to inflammasome assembly. (V) Upon activation, GSDMD may form pores in granule membranes, facilitating the release of proteases such as elastase and MMP-9, which could contribute to the cleavage of pro-IL-1β and GSDMD, ultimately promoting the release of IL-1β, elastase, MPO, and MMP-9. (VI) NETs, along with proteases, are released following NLRP3 activation, although the GSDMD dependency of this process is debated.

Nlrp3 –/– mice recruited fewer neutrophils to the peritoneum compared with *Nlrp3* +/+ mice, and showed lower IL-1β levels in the lavage fluid. IL-1β was thought to originate from neutrophils, as neutrophil depletion reduced IL-1β levels. Neutrophils from the peritoneum also formed more ASC specks. Endothelial cells isolated from mesenteric vessels in *Nlrp3* KO mice showed a lower percentage of *P*-selectin and lower mean fluorescence intensity, which could also impair leukocyte transmigration.[67]

Recent work by Zou et al.[115] employed the NEUT-SFL parameter—derived from automated hematology analysis—as an indicator of neutrophil activation and maturity in septic patients. Higher NEUT-SFL values correlated with increased NET formation and markers of endothelial damage. Using purified NETs from patient samples, the authors demonstrated significant NET-induced endothelial injury both in vitro and in murine models of sepsis. Importantly, these findings were directly associated with dysregulated NLRP3 inflammasome activation in neutrophils, linking NLRP3-dependent NET formation to vascular injury and systemic inflammation.

In a mouse model of myocardial infarction, transfusion with *Nlrp3* –/– neutrophils resulted in reduced infarct size and preserved left ventricular function. This was associated with decreased deposits of H3Cit-positive NETs and lower levels of IL-1β in myocardial tissue and plasma samples compared with wild-type (WT) mice.[116]

Similarly, in studies related to vaccine-induced thrombotic thrombocytopenia (VITT), VITT plasma was shown to induce formation of platelet–neutrophil aggregates in a FcγRIIa-dependent manner. These platelet–neutrophil interactions may contribute to thromboinflammation in VITT patients by supporting NLRP3 inflammasome activation.[117]

6.2 Cancer

Even in malignant conditions, such as oral squamous cell carcinoma (OSCC), significant enrichment of NETs has been reported and correlated with poor prognosis.[118] Neutrophil elastase (NE) within NETs appears to play a central role in modulating NLRP3 inflammasome activity and pyroptosis in OSCC cells, thereby favoring tumor invasion and metastasis. However, this atypical inhibitory effect of NE on inflammasome activation remains poorly explored. Beyond OSCC, chronic inflammation–associated malignancies such as ulcerative colitis and colorectal cancer also exhibit strong neutrophil infiltration and dysregulated

inflammasome signaling. In these contexts, aberrant NLRP3 activation can contribute to tissue damage, epithelial transformation, and tumor-promoting microenvironments,[119] suggesting that the interplay between neutrophils, NET components, and inflammasome pathways may be a common mechanism linking chronic inflammation to cancer progression.

6.3 Infections

An increasing body of evidence indicates that dysregulated NLRP3 activation in neutrophils contributes to a state of hyperinflammation associated with poor clinical outcomes not only in bacterial but also in viral infections such as SARS-CoV-2.[59,119] Consistent with the mechanisms described for bacterial pathogens—including *P. aeruginosa*,[32] *S. aureus*,[33] and *Str. pneumoniae*[29,30,34]—neutrophils can be primed through distinct pattern-recognition receptors (TLR2, TLR4, NOD2), depending on their anatomical or experimental origin, highlighting the contextual diversity of neutrophil inflammasome licensing. In addition to canonical NLRP3 activation, neutrophils also exhibit a functional noncanonical pathway in which cytosolic LPS triggers caspase-11 and caspase-1 activation, resulting in IL-1β secretion, pyroptotic features, and NET extrusion, a mechanism also observed in *A. fumigatus* infection.[36,37,120]

A similar inflammasome-centered signature has been characterized during viral infections. In severe SARS-CoV-2 disease, peripheral blood and lung neutrophils display enhanced NLRP3 activation, leading to elevated IL-1β production, increased NET release, and an imbalance in protease liberation (MPO, MMP-9), which correlates with markers of acute respiratory distress syndrome and cytokine storm severity.[57,72,119] Likewise, during Dengue virus (DENV) infection, the domain III of the viral envelope protein is sufficient to induce NETosis both in vitro and in vivo through NLRP3- and caspase-1-dependent mechanisms, contributing to systemic hyperinflammation.[62]

Taken together, these findings reinforce the concept that neutrophil inflammasome hyperactivation represents a central inflammatory axis in infectious diseases, integrating bacterial, fungal, and viral triggers into a shared immunopathological outcome.

7. Concluding remarks

Over the past decade, our understanding of NLRP3 inflammasome activation has expanded considerably beyond the classic models of monocytes and macrophages. Neutrophils, as the most abundant circulating leukocytes, have emerged as key players in inflammasome biology, exhibiting distinct regulatory mechanisms and functional outcomes. From IL-1β release to NET formation, neutrophil-specific features of NLRP3 activation include unique proteases, posttranslational regulators such as BTK, PAD4, PARP-1, and even alternative routes of GSDMD-mediated activity that may uncouple cytokine secretion from pyroptosis and skew the response toward PMN-specific NET release. These unconventional aspects provide new insight into how neutrophils contribute to inflammatory amplification, tissue damage, and immunopathology across a broad spectrum of diseases—including autoimmune, infectious, cardiovascular, and malignant conditions.

Importantly, the dual nature of neutrophils—both as initiators and amplifiers of inflammation—demands a deeper investigation of their inflammasome regulation. Targeting neutrophil-specific NLRP3 pathways may offer novel therapeutic opportunities. Future research must continue to define the molecular switches governing this compartmentalized activation, the intercellular crosstalk triggered by NET-associated components, and the translational implications of modulating inflammasome responses in neutrophils. Unlocking this knowledge may be essential to achieving more precise and effective therapies.

Acknowledgments

The authors thank Ms Libera Lo Presti for her valuable assistance in the revision of the manuscript, especially for her precise and thoughtful support with the English language.

Author contributions

V.N.C.L.: conceptualization and investigation. V.N.C.L and A.N.R.W.: data curation, review, editing, and revision, and writing original draft.

Funding

The authors were supported by the São Paulo Research Foundation (FAPESP; grant number 2020/15323-3) and the National Council for Scientific and Technological Development (CNPq; grant number 446201/2024-3). A.N.R.W. has been funded through: Deutsche Forschungsgemeinschaft (German Research Foundation, DFG) grants SPP2416 Project We-4195/24-1 "Principles of extra- and intracellular chitin perception by mammalian pattern-recognition receptors", SPP2225 Project We-4195/25-1 "STAPHEX—Dissecting Staphylococcus aureus macrophage exit", CRC TR156 "The skin as an immune sensor and effector organ—Orchestrating local and systemic immunity" INST 35/1259-2 and INST 35/1259-3 and a Volkswagen Stiftung Momentum Grant "InnatelyHuman". Infrastructural funding was provided by the University of Tübingen, the University Hospital Tübingen, and the DFG Clusters of Excellence "iFIT—Image-Guided and Functionally Instructed Tumor Therapies" (EXC 2180), "CMFI—Controlling Microbes to Fight Infection" (EXC 2124). Mandatory citation: "Gefördert durch die Deutsche Forschungsgemeinschaft (DFG) im Rahmen der Exzellenzstrategie des Bundes und der Länder—EXC-2180 und EXC-2124).

Conflicts of interest: None declared.

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