The NLRP3 inflammasome-extracellular vesicles axis: an emerging network in immune regulation

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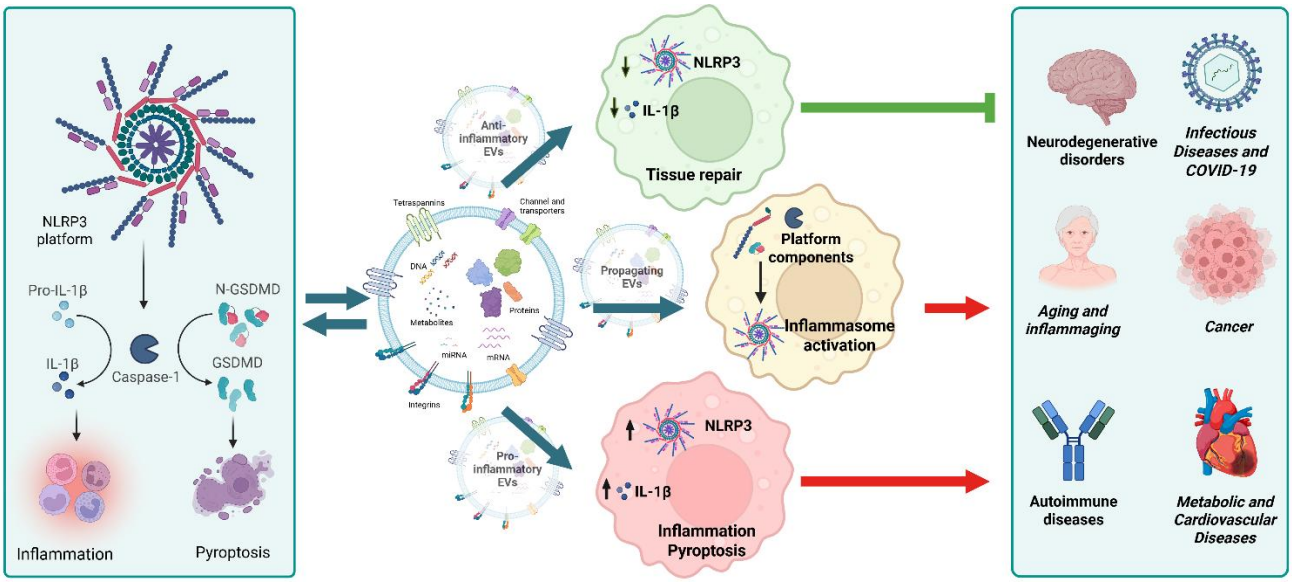
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Abstract

Immune homeostasis depends on the tightly coordinated activity of immune cells, soluble mediators and intracellular signalling pathways that together limit pathological inflammation. Two systems have emerged as central nodes in this regulatory network: the NLRP3 inflammasome, a cytosolic sensor whose aberrant activation is a hallmark of inflammatory, autoimmune and age-related diseases, and extracellular vesicles (EVs), which transport a specific cargo that shapes the behaviour of the recipient cells. These systems have largely been studied separately, but accumulating evidence points to a reciprocal relationship. NLRP3 activation influences EV biogenesis, cargo composition and release, while EVs can prime, amplify or dampen inflammasome activation in recipient cells, extending inflammatory signalling beyond the cell of origin. In this review, we bring these two lines together and examine how NLRP3 inflammasome activity and EV-mediated communication intersect to regulate immune cell function, cytokine release and the propagation of inflammation. We discuss the mechanisms supporting this bidirectional crosstalk, highlight open questions and current limitations, and consider how targeting the NLRP3-EV axis might open new therapeutic opportunities. Viewing inflammasome activation and EV-mediated signalling as an integrated circuit, rather than as separate pathways, may promote the understanding of how local inflammatory events expand, and can lead to the development of innovative strategies to control chronic inflammation.



Graphical abstract. Role of the NLRP3 inflammasome platform and extracellular vesicles (EVs) in inflammation regulation and disease. Left box: Activation of the NLRP3 platform leads to caspase-1 activation, which processes pro-IL-1β into its active form (IL-1β) and cleaves gasdermin D (GSDMD), resulting in pyroptosis and in the release of inflammatory mediators. EVs carry proteins, nucleic acids, and metabolites that mediate intercellular communication and propagate inflammasome-related signals between cells. EV-mediated signalling can exert multiple effects. Anti-inflammatory EVs reduce NLRP3 activation and IL-1β production, thus promoting tissue repair. In contrast, pro-inflammatory EVs enhance NLRP3 activation and IL-1β secretion, thus amplifying inflammation. Red arrows indicate a positive contribution, green blunted arrows represent modulation of various conditions, including neurodegenerative disorders, infectious diseases (e.g., COVID-19), ageing-associated inflammation (inflammageing), cancer, autoimmune diseases, and metabolic and cardiovascular disorders. (Created in BioRender. Bellezza, I. (2026) https://BioRender.com/2r4qfng)

Introduction

Innate immunity represents the host's non-specific first line of defence against pathogens and plays a central role in shaping the subsequent specific adaptive immune responses. Innate immunity relies on specific proteins known as pattern-recognition receptors (PRRs), which interact with molecules associated with infection or damage. PRRs are generally classified into five distinct families: Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs), and absent in melanoma 2 (AIM2)-like receptors (ALRs), which sense endogenous and exogenous danger signals. PRRs detect and bind pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and trigger intracellular signalling cascades aimed at restoring homeostasis. PRR signalling promotes the transcription of genes involved in inflammatory responses, including cytokines and chemokines, thereby contributing to innate immune responses and to the development of adaptive immunity [1,2]. Among these mediators, interleukin-1β (IL-1β) is a major pro-inflammatory cytokine, involved in host defence, inflammatory responses, and tissue remodelling [3,4]. IL-1β production requires two sequential steps: first, PRR signalling induces the synthesis of the inactive precursor pro-IL-1β; subsequently, inflammasome assembly triggers caspase-1 activation, leading to the proteolytic processing of pro-IL-1β into its mature, biologically active form [5].
After pathogen clearance, endogenous regulatory mechanisms limit the inflammatory response and contribute to the restoration of tissue homeostasis, thus preventing excessive tissue damage [6]. An important contributor to this regulatory process is represented by extracellular vesicles (EVs) [7-9]. EVs are membrane-enclosed particles released by virtually all cell types that mediate intercellular communication. Several studies indicate that EVs can regulate multiple checkpoints of immune responses and NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in recipient cells [7-12]. The reciprocal interaction between EVs and the NLRP3 inflammasome is therefore relevant to both immune regulation and inflammatory disease [9,13-18].

NLRP3 inflammasome structure

The inflammasomes are cytoplasmic signalling platforms that sense exogenous and endogenous danger signals and coordinate cytokine release and pyroptotic cell death. Inflammasome activation leads indeed to the maturation of the pro-inflammatory cytokines IL-1β and IL-18 and to the cleavage of gasdermin D (GSDMD), directly responsible for the induction of pyroptotic cell death [3,19-21]. The ability of inflammasomes to recognize a wide range of danger signals relies on the presence of several different intracellular receptors, among which the NLR family is the most studied and best characterized. It comprises several members including NLRP1, NLRP3, NLRP6, and NLRC4 each of which is activated by different PAMPs. For example, NLRP1 is activated by viral proteases and virus-associated nucleic acids including double-stranded RNA, NLRP6 is activated by lipoteichoic acid, a structural component of the Gram-positive bacteria cell wall, and NLRC4 responds to bacterial flagellin [3,22,23]. Notably, other intracellular PRRs are capable of inducing inflammasome activation, for instance AIM2, by recognizing double-stranded DNA, induces inflammasome-mediated IL-1β maturation [3,20,21,23,24]. Among the different inflammasomes, NLRP3 is the best characterized and the most extensively studied. It consists of three major components: (i) the cytoplasmic receptor NLRP3, which serves as sensor for the recognition of cellular perturbations and for the recruitment of downstream molecules; (ii) the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), which functions as a bridge connecting NLRP3 and pro-caspase-1; and (iii) the effector enzyme pro-caspase-1, whose activation prompts the maturation of IL-1β and IL-18 and the cleavage of GSDMD [19,21,23,24].
The NLRP3 receptor has a tripartite domain organization: (i) the N-terminal pyrin domain (PYD), involved in ASC recruitment via PYD-PYD interaction; (ii) the central NTPase domain (NACHT), carrying ATPase activity essential for NLRP3 activation and platform assembly; and (iii) the C-terminal leucine-rich repeats (LRRs), responsible for autoregulation and protein-protein interactions [25-27] (**Fig. 1**). The adaptor protein ASC comprises two primary domains (PYD and CARD - caspase activation and recruitment domain) connected by a flexible linker sequence; upon activation, ASC self-oligomerizes into filamentous scaffolds known as "ASC specks" that enable efficient pro-caspase-1 recruitment via CARD-CARD interactions [25].

The deciphering of these structural insights provided the basis for the development of selective modulators of inflammasome activation.

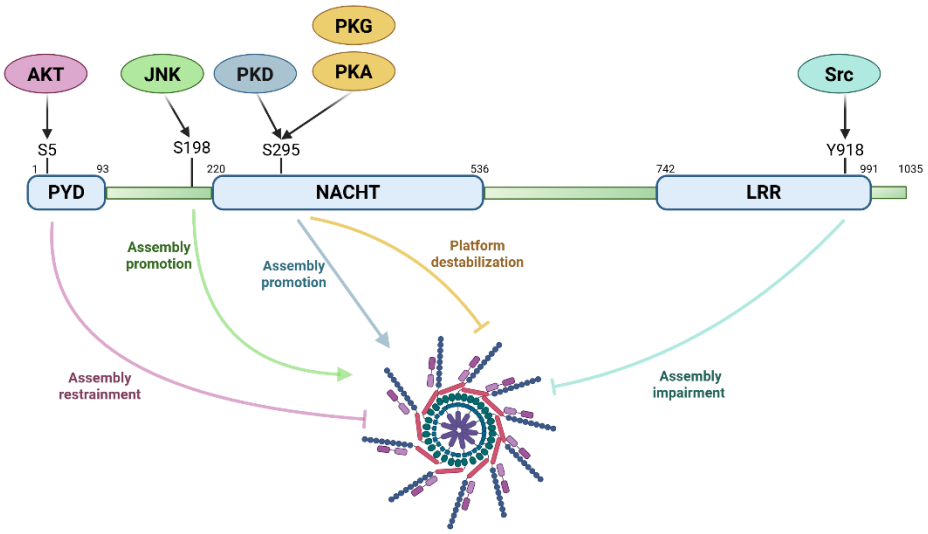


Figure 1. NLRP3 domain organization and its regulation by phosphorylation. Schematic representation of NLRP3 protein (UniProt: Q96P20) showing the N-terminal pyrin domain (a. 1-93), central NACHT domain (aa 220-536), and C-terminal LRR domain (aa 742-991). The phosphorylated residues and the acting protein kinases are indicated above. The effect of phosphorylation is depicted below by colour-corresponding arrows. JNK and PKD promote NLRP3 activation by phosphorylating Ser198 and Ser295, respectively. AKT inhibits NLRP3 homomultimerization by phosphorylating Ser5, PKA and PKG inhibit NLRP3 activity by acting on Ser295, Src negatively regulates NLRP3 inflammasome assembly via Tyr918 phosphorylation. (Created in BioRender. Bellezza, I. (2026) https://BioRender.com/ogflew1).

Molecular mechanisms of canonical NLRP3 inflammasome activation

In the resting state, NLRP3 assumes a "double-ring" or "cage-like" conformation composed of 12 to 16 NLRP3 monomers in which PYD are sequestered within the assembly, thus preventing spontaneous inflammasome activation [28]. Upon sensing danger signals, NLRP3 recruits NIMA-related kinase 7 (NEK7), which promotes conformational changes rearranging the NLRP3 cage into a disc composed of 10 to 11 NLRP3 monomers. The acquisition of the disc conformation is pivotal for ASC recruitment and caspase-1 binding. Once recruited, pro-caspase-1 undergoes proximity-induced auto-activation, forming active caspase-1 responsible for IL-1β/IL-18 maturation and GSDMD cleavage [21,25,28-30].

NLRP3 can be activated through three mechanistically distinct modes: canonical, non-canonical, and alternative [31-33]. Among these, the canonical pathway is the most extensively characterized and requires two sequential steps: priming and activation/assembly [31]. During priming, stimulation of PRRs, such as TLR4, drives nuclear factor kappa B (NF-κB)-dependent transcriptional upregulation of inflammasome components, pro-IL-1β and pro-IL-18 [25,27,34,35]. The activation step is triggered by intracellular PAMPs or DAMPs, which promote inflammasome assembly leading to caspase-1 activation. Activated caspase-1, in turn, cleaves pro-IL-1β and pro-IL-18 into their active/mature forms and induces GSDMD limited proteolysis [21,24,26,36]. Both priming and assembly are controlled by structural changes and post-translational modifications (PTMs) [21,24,26,36], the latter providing an additional level of control of canonical NLRP3 activation [36-40]. Reversible PTMs regulate NLRP3 protein stability, conformational flexibility, intracellular trafficking, and interactions with adaptor proteins [36]. Ubiquitination can either promote inflammasome assembly or target NLRP3 for degradation, depending on the type of ubiquitin chain, whereas deubiquitinating enzymes reverse these effects [41,42]. Acetylation generally enhances NLRP3 activation by promoting its oligomerization, while deacetylases, including sirtuins and histone deacetylases, act as negative regulators of inflammasome signalling and can influence NLRP3 transcription through epigenetic control mechanisms [41,43]. Lipid modifications, including palmitoylation, play important roles in controlling NLRP3 intracellular

localization and promote its interaction with NEK7 and ASC, thereby facilitating inflammasome assembly [41,44]. Additional PTMs, such as SUMOylation, may influence NLRP3 stability in a context-dependent manner [44,45]. The conversion of arginine into citrulline by peptidyl arginine deiminases has also been associated with enhanced inflammasome assembly and increased IL-1β release [41,46]. Among these PTMs, phosphorylation is particularly important because it regulates NLRP3 conformation, stability, and interactions at different stages of inflammasome activation.

Regulation of inflammasome priming/assembly by phosphorylation networks

Reversible phosphorylation events regulate NLRP3 activity during both priming and inflammasome assembly (**Fig. 1**). These phosphorylation events can increase or decrease the threshold for inflammasome activation [47].

During the priming phase, lipopolysaccharide (LPS), by binding TLR4, not only upregulates the transcription of NLRP3 inflammasome components, but also stabilizes NLRP3 protein by limiting FBXL2 (F-box/LRR-repeat protein 2)-mediated ubiquitination and proteasomal degradation, thereby increasing the pool of activation-competent NLRP3 [48]. Priming also engages phosphorylation-dependent mechanisms that finely regulate NLRP3 activation. For example, LPS activates AKT (RAC-alpha serine/threonine-protein kinase), which phosphorylates NLRP3 at serine 5 (S5), preventing the E3 ubiquitin-protein ligase TRIM31-driven ubiquitination at lysine 496 (K496) and protecting NLRP3 from degradation [47]. Phosphorylation at S5 provides another layer of control, as it also prevents premature NLRP3 oligomerization when a secondary activation signal is absent. In this way, the inflammasome remains in a primed but inactive state [47,49]. When activating stimuli occur, protein phosphatase 2A (PP2A) removes the phosphate group from S5, enabling proper inflammasome assembly. This dephosphorylation may also restore TRIM31 ubiquitin ligase activity and promote NLRP3 degradation [47]. Phosphorylation at this N-terminal-proximal residue also modulates the interaction between NLRP3 and the adaptor protein ASC, suggesting that these early phosphorylation events regulate conformational accessibility and adaptor recruitment rather than directly prompting NLRP3 activation [47,49].

Another key regulatory site is S295, located in the NACHT domain. Phosphorylation of S295 can exert different effects depending on the kinase involved. Phosphorylation by protein kinase A (PKA) attenuates the ATPase activity of NLRP3 [50], while phosphorylation by cGMP-dependent protein kinase I (PKG-I) reduces inflammasome complex formation [51]. In contrast, phosphorylation of the same S295 residue by protein kinase D (PKD) promotes NLRP3 oligomerization and inflammasome activation. This apparent paradox supports a model in which coordinated phosphorylation-dephosphorylation cycles strictly control NLRP3 activation [38,47].

Additional inhibitory phosphorylation events further restrict inflammasome activation. For example, Src family kinase members, including Lyn, phosphorylate NLRP3 at tyrosine 918 (Y918), impairing inflammasome assembly and reducing IL-1β maturation [52]. In contrast, phosphorylation of S198, located within an “activation loop” at the interface between the NACHT and LRR domains, occurs early during priming and is required for efficient NLRP3 activation mediated by c-Jun N-terminal kinase 1 (JNK1) [38,47]. Additional studies have expanded this phospho-regulatory framework by identifying further phosphorylation sites including Y32, S163, threonine 193 (T193), T195, and S216, that contribute to the regulation of NLRP3 assembly at different stages [47,53].

During priming, upstream kinases, such as inhibitor of nuclear factor kappa-B kinase subunit alpha (IKKα), contribute to negative regulation of the NLRP3 inflammasome by phosphorylating ASC [54,55]. Subsequent phosphatase-mediated dephosphorylation removes this inhibitory checkpoint, allowing NLRP3 oligomerization and inflammasome activation in response to appropriate danger signals [47].

NLRP3 phosphorylation is therefore not uniformly activating or inhibiting; its functional effect depends on the residues modified, the kinases and phosphatases involved, and the stage of inflammasome activation.

Regulation of inflammasome assembly by diverse cellular stress pathways

The activation step (signal 2) is triggered by a variety of cellular perturbations including potassium (K^+) efflux, calcium (Ca^{2+}) mobilization, lysosomal damage, and mitochondrial dysfunction (**Fig. 2**) [20,21,25,26,31,56]. K^+ efflux is one of the best characterized upstream events in NLRP3 activation and is induced by many inflammasome activators including (i) potassium-proton ionophores such as nigericin; (ii) ATP binding to the P2X7 receptor driving cation influx and K^+ efflux via the tandem of P domains in a weak inward rectifying K^+ channel 2 (TWIK2); (iii) particulate matter such as environmental particles and crystals that can directly induce K^+ efflux [26,57,58].

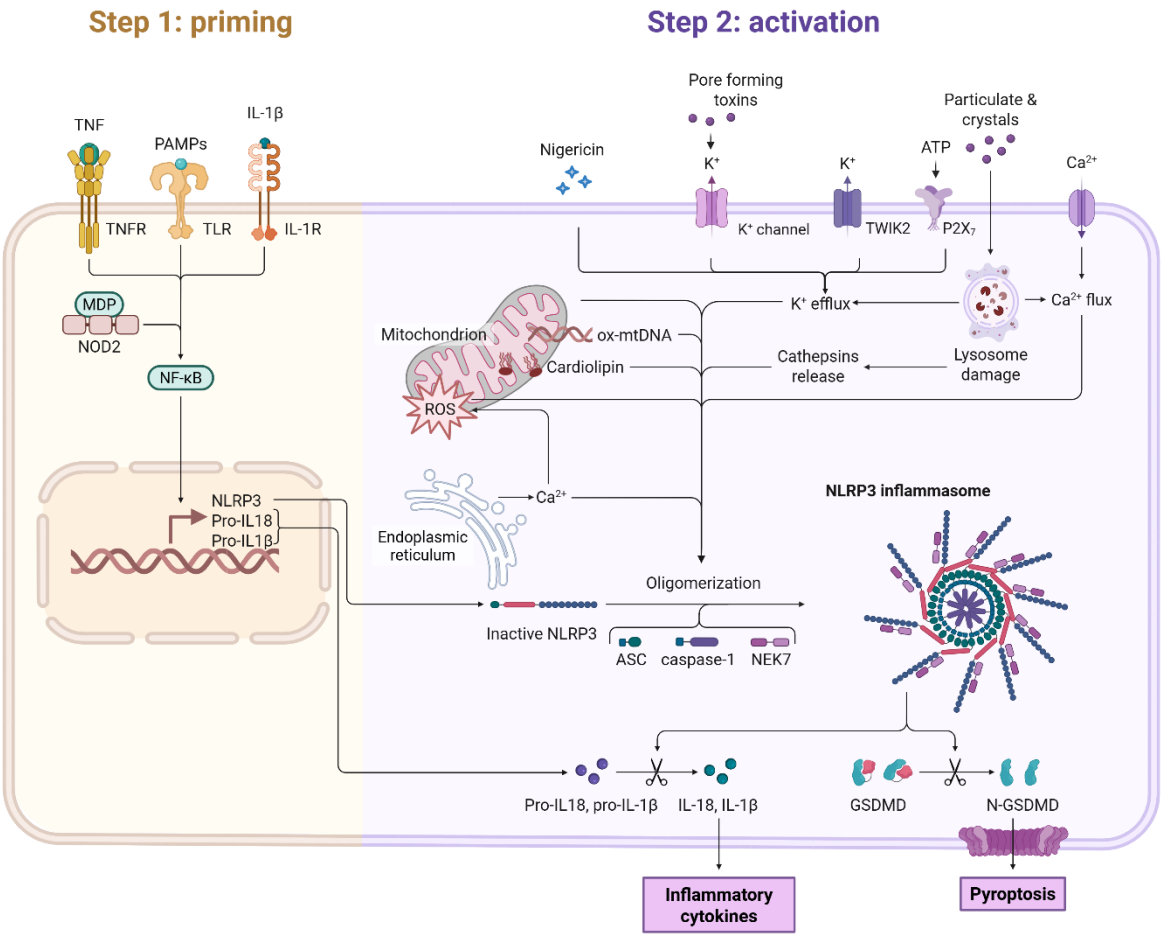


Figure 2. The two-step activation process of the NLRP3 inflammasome. Schematic representation of the canonical NLRP3 inflammasome activation pathway.
Priming (Step 1): Recognition of pathogen-associated molecular patterns (PAMPs) and pro-inflammatory cytokines by pattern-recognition receptors (PRRs) and cytokine receptors causes NF-κB activation. NF-κB upregulates the expression of NLRP3 inflammasome components and of the full-length pro-inflammatory cytokines (pro-IL-1β and pro-IL-18). Activation (Step 2): A variety of stress signals trigger NLRP3 inflammasome assembly. These include ion fluxes (K^+ efflux, Ca^{2+} mobilization), ATP stimulation via P2X7 receptors, pore-forming toxins, crystalline and particulate matter, lysosomal destabilization with cathepsin release, and mitochondrial dysfunction with generation of reactive oxygen species (ROS), the release of oxidized mitochondrial DNA (ox-mtDNA), and cardiolipin exposure. The release of endoplasmic reticulum- and lysosome-stored Ca^{2+} further contributes to NLRP3 inflammasome assembly/activation.
These events promote NLRP3 oligomerization and recruitment of the adaptor protein ASC and pro-caspase-1, leading to the formation of the NLRP3 inflammasome platform which promotes the cleavage/activation of pro-caspase-1. Active caspase-1, in turn, cleaves pro-IL-1β and pro-IL-18 into their mature forms and processes gasdermin D (GSDMD) to generate the pore-forming N-terminal fragment (N-GSDMD). Modified and created with BioRender.com by Bellezza I. Adapted from "NLRP3 Inflammasome Activation" by BioRender.com, 2026. Retrieved from https://BioRender.com/vxuwyes.

An increase in cytosolic Ca^{2+} is another important signal promoting NLRP3 activation [59,60]. Elevated cytosolic Ca^{2+} can be induced by ATP stimulation, pore-forming toxins, viral infection, crystalline particles, or by Ca^{2+} release from intracellular stores, including the endoplasmic reticulum and lysosomes, as well as from extracellular Ca^{2+} influx through plasma membrane channels [59,60]. Increased Ca^{2+} levels can promote NLRP3 activation in several ways. It can directly facilitate conformational changes within the NACHT domain, reduce intracellular cAMP levels, thereby limiting PKA-mediated inhibitory signalling [59,60], or promote mitochondrial Ca^{2+} overload, leading to mitochondrial dysfunction, mitochondrial reactive oxygen species (mtROS) generation, mitochondrial DNA (mtDNA) release, and cardiolipin externalization [61].
Mitochondrial dysfunction can also promote NLRP3 activation through several mechanisms. When mitochondria become dysfunctional, mtDNA can leak into the cytosol and mitochondrial reactive oxygen species (mtROS) accumulate, both are well-established triggers of NLRP3 activation. At the same time, alteration of mitochondrial membrane potential promotes the recruitment of NLRP3 to mitochondria-associated membranes, a key step that supports inflammasome assembly [62].
Mitochondrial stress signalling is also linked to lipid remodelling. Cardiolipin, a phospholipid mainly localized in the inner mitochondrial membrane, under oxidative stress conditions, undergoes oxidation and externalization, contributing to mitochondrial dysfunction [63]. Once exposed on the outer mitochondrial membrane, cardiolipin can directly interact with NLRP3 and promote inflammasome platform assembly and activation [64].
Another major mechanism leading to NLRP3 activation is lysosomal damage. Poorly degradable particles, including monosodium urate crystals, cholesterol crystals, and amyloid-β aggregates, may accumulate within phagolysosomes. Their accumulation promotes lysosomal swelling and ultimately compromises membrane integrity, causing lysosomal rupture. The consequent release of lysosomal proteases, particularly cathepsins, into the cytosol promotes NLRP3 activation [65].
These different forms of cellular stress can therefore converge on NLRP3 inflammasome activation.
Once assembled, the NLRP3 inflammasome acts as a signalling scaffold that recruits and activates pro-caspase-1 which processes pro-IL-1β and pro-IL-18 into their active forms [4,5]. Caspase-1 also cleaves GSDMD, initiating pyroptosis. This cleavage releases the pore-forming N-terminal fragment (N-GSDMD), which oligomerizes in the plasma membrane to form large transmembrane pores that mediate the secretion of IL-1β and IL-18 while progressively disrupting membrane integrity. These events culminate in pyroptotic cell death [66,67].
N-GSDMD pores also promote K^+ efflux, cell swelling, and eventually plasma membrane rupture, leading to the release of intracellular DAMPs that further amplify and propagate inflammatory signalling within the surrounding tissue [68]. However, inflammasome activation does not always culminate in immediate cell lysis. Under certain conditions, particularly in macrophages, GSDMD pores allow the release of IL-1β while maintaining temporary cell viability, generating a transient hyperactivated state before terminal pyroptosis occurs [69].
Collectively, these mechanisms regulate NLRP3 activation at different stages, from priming to inflammasome assembly and downstream signalling to permit a proper cellular response to danger while preventing unwarranted inflammasome activation.

Biological role of the NLRP3 inflammasome in immune regulation

NLRP3 is expressed in several immune and immune-related cell types where it participates in host defence, immune homeostasis, and inflammatory responses. Through IL-1β and IL-18 maturation and release, NLRP3 can influence both innate and adaptive immunity [70-72]. Neutrophils, peripheral blood mononuclear cells (PBMCs), and bone marrow-derived dendritic cells constitutively express relatively high levels of NLRP3, whereas macrophages and Th2 cells show a lower basal expression that increases through NF-κB signalling following TLR or TNF receptor stimulation [73-76].
IL-18 links NLRP3 inflammasome activation to Th1-polarized T cells-mediated immune response. Mature IL-18 can indeed promote the production and release of IFN-γ by T lymphocytes and natural killer cells, supporting further Th1 polarization and macrophage activation [77,78]. Although Th1 immunity is essential

for cell-mediated host protection, excessive or dysregulated Th1 responses can contribute to autoimmune pathology [79].
In experimental autoimmune encephalomyelitis, NLRP3 promotes pathogenic Th1 and Th17 responses and contributes to central nervous system (CNS) inflammation through IL-18-dependent mechanisms [79]. However, disease progression can also occur in NLRP3-deficient animals, indicating that NLRP3-independent mechanisms can also contribute to autoimmune neuroinflammation in different experimental models of disease [80]. Inflammasome signalling modulates regulatory T-cell differentiation and stability also through IL-1β-dependent control of regulatory T (Treg) cells suppressive function [81,82]. Moreover, IL-1β contributes to the recruitment and activation of neutrophils and inflammatory monocytes at sites of infection or tissue injury and can enhance macrophage phagocytic activity and inflammatory mediator production [3,70].
NLRP3 can also influence adaptive immunity through antigen-presenting cells, including dendritic cells [83]. Following LPS/ATP stimulation, conventional dendritic cell subsets show different levels of NLRP3 activation, consistent with their distinct functional roles [84]. NLRP3 also participates in trained immunity through epigenetic and metabolic remodelling of myeloid cells [85-87].
In addition, NLRP3 also contributes to tissue homeostasis. In barrier tissues such as the gut and the CNS, controlled inflammasome activation can support epithelial integrity, pathogen clearance, and tissue repair, whereas persistent activation can promote chronic inflammation and tissue damage [88-91].
Therefore, a tight control of NLRP3 activation is essential to ensure a proper inflammasome response necessary to guarantee tissue homeostasis.

Dysregulation of the NLRP3 inflammasome and disease

Persistent NLRP3 inflammasome activation is associated with several chronic diseases, including cardiovascular disorders, metabolic diseases such as type 2 diabetes and obesity, neurodegenerative conditions including Alzheimer's and Parkinson's diseases, cancer, and severe COVID-19 [89-98]. Genetic alterations, chronic metabolic stress, environmental stimuli, and age-associated changes can disrupt the mechanisms that normally restrict NLRP3 activity, leading to sustained caspase-1 activation, increased IL-1β and IL-18 production, pyroptosis, chronic immune activation with progressive tissue injury and loss of immune tolerance [88-100].
The pathogenic potential of inflammasome dysregulation is illustrated by gain-of-function mutations in the NLRP3 gene, which mutations cause cryopyrin-associated periodic syndromes (CAPS), including familial cold autoinflammatory syndrome, Muckle-Wells syndrome, and neonatal-onset multisystem inflammatory disease. These disorders are characterized by constitutive inflammasome activation, abnormal IL-1β and IL-18 production, and chronic systemic inflammation [89-97]. The clinical efficacy of IL-1-blocking therapies in CAPS supports the central role of dysregulated NLRP3 signalling in chronic inflammatory disease [88-101].

Autoimmune and immune-mediated diseases

In more common polygenic and environmentally conditioned disorders, NLRP3 dysregulation results from persistent innate immune activation rather than from single-gene mutations. In autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, increased NLRP3 expression and inflammasome activation are associated with disease activity, autoantibody production, and inflammation-mediated tissue damage [89,99,101].
In organ-specific immune-mediated diseases, particularly in experimental models of autoimmune encephalomyelitis and type 1 diabetes, NLRP3 activation promotes pro-inflammatory Th1 and Th17 differentiation, enhances antigen-presenting cell activation, and sustains chronic inflammation within target tissues [79,80,95,99,101,102]. A similar role has been described in chronic intestinal inflammatory disorders such as inflammatory bowel disease, where NLRP3-dependent maturation of IL-1β and IL-18 amplifies mucosal immune responses and contributes to epithelial damage [103]. However, growing evidence also suggest that inflammasome signalling can also exert protective effects in the gut, by maintaining epithelial barrier integrity and supporting intestinal immune homeostasis [89,97,101].

Neurodegenerative disorders

NLRP3 activation contributes to sterile neuroinflammation in Alzheimer's and Parkinson's diseases, where inflammasome-derived cytokines exacerbate neuronal injury [79,95,96]. In Alzheimer's disease, amyloid-β activates inflammasome signalling and IL-1β release in microglia, similarly α-synuclein aggregates stimulate inflammasome activation in Parkinson's disease [95]. Oxidative stress can also promote NLRP3 activation in the CNS. Supporting the contribution of redox imbalance to inflammasome-dependent neuroinflammation, pharmacological reduction of oxidative stress suppresses NLRP3 activation in mutant superoxide dismutase 1 microglia, an experimental model of amyotrophic lateral sclerosis [104]. Microglia are the principal source of inflammasome activity in the CNS, although astrocytes and, under some conditions, neurons can also contribute, increasing the complexity of neuroinflammatory responses. The effects of persistent inflammasome activation and abnormal cytokine production are at the basis of synaptic dysfunction and neuronal injury. Accordingly, chronic low-grade inflammasome activation in microglia may induce cytokine release that sustains a pro-inflammatory microglial state in the CNS. This vicious cycle has been associated with age-related neuroinflammation, known as "neuro-inflammageing", which contributes to memory loss and cognitive decline in the elderly [105]. These findings suggest that dysregulated innate immunity may represent a common mechanism linking ageing to the development and progression of neurodegenerative diseases.

Infectious diseases and COVID-19

During infection, PAMPs and host-derived danger signals activate NLRP3 in myeloid cells, leading to caspase-1 activation, IL-1β and IL-18 maturation, and, in some settings, pyroptotic cell death. These responses contribute to antimicrobial defence but can cause tissue damage when excessive or prolonged [3,5,20,22]. Also neutrophils participate in inflammasome-dependent responses during infection although with unconventional mechanisms [106]. First, the amount of NLRP3-dependent IL-1β released by individual neutrophils is relatively low if compared with macrophages. However, during immune responses, large numbers of neutrophils are recruited to sites of infection potentially compensating for the smaller amount of IL-1β produced [106]. In addition, neutrophils may release IL-1β more rapidly than macrophages. Moreover, although microbial toxins can directly activate canonical and non-canonical NLRP3 pathways, neutrophils do not undergo pyroptosis. This may be explained by the evidence that caspase-1 processes GSDMD less efficiently than pro-IL-1β, maintaining neutrophils in a sublytic state, allowing them to release IL-1β without undergoing pyroptotic cell death [106]. Interestingly, following activation of the non-canonical NLRP3 inflammasome, GSDMD maturation plays an important role in inducing NETosis [106], a process in which neutrophils release a network of DNA and proteins that helps trap and eliminate invading microorganisms [107].

Several RNA viruses, including influenza viruses and coronaviruses, activate NLRP3 through ionic imbalance, mitochondrial stress, and ROS production [108]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins, including ORF3a and the E protein, can promote K^+ efflux and lysosomal damage, facilitating NLRP3 activation in infected and neighbouring cells, supporting the early antiviral immune response [109]. In severe COVID-19, sustained NLRP3 activation in circulating and tissue-resident immune cells is associated with excessive IL-1β and IL-18 release, contributing to the hyperinflammatory state associated with acute respiratory distress syndrome, multi-organ involvement, and poor clinical outcomes [98,109,110]. Inflammasome activation is also observed in epithelial and endothelial cells, where it can contribute to vascular barrier dysfunction, pro-coagulant activity, and microvascular inflammation [98,109,110]. Moreover, platelets from COVID-19 patients display an increase in ROS production associated with NLRP3 activation [111]. It is to highlight that pyroptotic cells can release DAMPs and inflammasome-associated components that amplify immune activation and tissue injury. Dysregulated inflammasome activity may also have consequences beyond the acute phase. Persistent NLRP3 signalling has been implicated in post-acute COVID-19, where prolonged innate immune activation may contribute to chronic inflammation and neuroimmune alterations [98,109,110], indicating that excessive NLRP3 activation can be detrimental during infection.

Metabolic and cardiovascular diseases

Increasing evidence indicates that metabolic and lifestyle-associated disorders can be considered immune-mediated diseases in which chronic NLRP3 inflammasome activation leads to persistent sterile inflammation [90,102,112,113]. In obesity and type 2 diabetes, visceral adipose tissue, hyperglycaemia, and hypercholesterolaemia are associated with tissue inflammation and provide persistent NLRP3 activating signals for myeloid and tissue-resident immune cells. Indeed, obesity promotes NLRP3 and macrophage-mediated T-cell and endothelial cell activation through the release of adipokines by the adipose tissue. In these conditions, NLRP3 activation in adipose tissue contributes to insulin resistance, β-cell dysfunction, adipose tissue chronic inflammation and impaired glucose homeostasis [90,114].
In gout and atherosclerosis, monosodium urate and cholesterol crystals, acting as crystalline DAMPs, activate NLRP3 and promote IL-1β-dependent inflammation in joints and vascular plaques promoting tissue injury [88,99]. Western-type diets, excess nutrient intake, and sedentary behaviour can also drive epigenetic and metabolic reprogramming of myeloid cells, increasing inflammasome activation and sustaining chronic inflammation [90,115].
Chronic NLRP3 activation also contributes to cardiovascular complications associated with metabolic disorders, including atherosclerosis, myocardial infarction, and heart failure. In this context, sustained IL-1β and IL-18 production can promote endothelial dysfunction, abnormal cardiac remodelling, and myocardial fibrosis [90,94].

Ageing and inflammageing

Ageing represents a biological context in which chronic low-grade NLRP3 inflammasome activation contributes to the progressive loss of immune homeostasis, promoting inflammageing and immunosenescence [116]. During ageing, persistent inflammasome activation can impair tissue repair and increase susceptibility to age-related disorders, including metabolic, cardiovascular, and neurodegenerative diseases [116,117].
Mitochondrial dysfunction and the resulting oxidative stress are among the hallmarks of ageing [118,119] and represent important contributors to age-related inflammasome activation. In fact, ROS act as critical upstream triggers that activate NF-κB signalling, which can intervene in the priming step [25,27,34,35] while ROS have been involved in the activation steps of inflammasome activation [62].
This oxidative stress-senescence-inflammasome axis has also been implicated in age-related retinal degeneration [63].
Moreover, senescent cells undergo irreversible growth arrest and acquire a senescence-associated secretory phenotype (SASP), controlled by inflammasome-mediated IL-1β signalling [120]. The SASP, characterized by the release of cytokines, chemokines, growth factors, and matrix-remodelling enzymes, can in turn reshape the tissue microenvironment by increasing inflammation and inducing paracrine senescence in neighbouring cells [120].
Accumulating evidence further indicates that, although many autoimmune diseases arise relatively early in life, they often display molecular and cellular characteristics commonly associated with ageing, including telomere shortening, epigenetic remodelling, metabolic dysfunction, and altered immune cell differentiation. In this context, chronic NLRP3 inflammasome activation may drive premature immune ageing by sustaining IL-1 cytokine production, oxidative stress, and SASP-associated inflammation [116,121].
NLRP3-dependent inflammageing may therefore contribute to the development and persistence of chronic immune-mediated diseases during ageing. This perspective supports the concept that therapeutic strategies targeting inflammasomes, senescent cell accumulation (senolytics), or SASP components could complement conventional immunosuppression, particularly in autoimmune settings where biological immune ageing may diverge from chronological age [121].

Cancer

NLRP3 inflammasome activation has been increasingly implicated in cancer, where it contributes to the complex interplay between chronic inflammation and tumour progression. Through IL-1β and IL-18, NLRP3 activation can affect both immune and stromal components of the tumour microenvironment. However, the

effects of NLRP3 activation can differ based on tumour type and stage, ranging from support of anti-tumour immunity to activation of inflammatory and immunosuppressive responses [27,122,123]. In myeloid and stromal cells, NLRP3-dependent IL-1β signalling can reduce cytotoxic T-cell activity, favour regulatory T-cell expansion, and support angiogenesis and tissue remodelling, ultimately facilitating tumour growth and metastasis [27,89,122,123]. Inflammasome signalling can also contribute to the recruitment and polarization of immune cells towards an immunosuppressive phenotype. On the other hand, during the early stages of tumour development inflammasome activation may support immune surveillance and tumour elimination [123].

NLRP3 signalling may also affect tumour cell-intrinsic processes, including proliferation, epithelial-mesenchymal transition, and invasion, partly through autocrine IL-1β signalling [123,124]. Furthermore, NLRP3 activity has also been associated with metabolic adaptation and resistance to chemotherapy and immunotherapy, supporting its contribution to tumour progression and treatment failure [122-124].

Thus, the contribution of NLRP3 to cancer is not uniform but varies according to tumour type, cellular context, and disease stage. This complexity should be considered when evaluating NLRP3 as a therapeutic target in cancer.

Therapeutic strategies targeting the NLRP3 inflammasome

Therapeutic targeting of the NLRP3 inflammasome has emerged as a promising strategy for the treatment of inflammatory and immune-mediated diseases. Current pharmacological approaches target different steps of the inflammasome pathway, ranging from NLRP3 activation to downstream effector mechanisms and cytokine production [27,56] (**Table 1**).

In particular, direct NLRP3 inhibition has been extensively investigated, and several small molecules have reached phase I/II clinical trials. MCC950 has been shown to suppress caspase-1 activation and IL-1β maturation in several inflammatory models, including experimental autoimmune encephalomyelitis [125], but its clinical development was discontinued because of hepatotoxicity [126]. DFV890 showed favourable pharmacokinetic and pharmacodynamic profiles in first-in-human studies [127], while RO7486967 (selnoflast) reached drug exposure levels expected to inhibit IL-1β, but biomarker data suggest that a clear treatment effect should not be expected [128]. OLT1177 (dapansutrile) reduced NLRP3 ATPase activity and prevented NLRP3 from interacting with ASC and caspase-1 and exerted anti-inflammatory effects on several preclinical models [129-131]. OLT1177 is undergoing several phase II clinical trials for inflammation-based diseases and a phase III clinical trial for acute gout flare (ClinicalTrials.gov).

AZD4144 is a potent and selective NLRP3 inhibitor that binds directly to NLRP3 and competes with MCC950 for binding, suggesting a shared mechanism that stabilizes the inactive conformation of NLRP3 and prevents inflammasome activation [132].

ML345 is a selective non-covalent inhibitor active in canonical, non-canonical, and alternative NLRP3 pathways. It disrupts a key interaction within the NACHT domain, preventing NEK7 binding and subsequent inflammasome assembly. It has also been shown to reduce inflammatory responses in mouse models of NLRP3-associated disorders [133]. Nimbolide, a natural compound derived from neem (*Azadirachta indica*), suppresses NLRP3-mediated inflammation in experimental colitis by disrupting NEK7-NLRP3 binding and impeding ASC oligomerization [134]. Phenstatin-based indole-chalcone hybrids, capable of binding the NLRP3 NACHT domain, are also being investigated as potential NLRP3 inhibitors [135]. Together, these compounds illustrate the ongoing development of selective strategies that directly target NLRP3 to prevent its activation.

Regulatory mechanisms upstream of NLRP3 activation also represent potential druggable targets. In this context, MF-094 inhibits the deubiquitinase ubiquitin-specific peptidase 30 (USP30) and reduces NLRP3 activation in a rat model of diabetic wound healing [136].

Downstream inflammasome components are also pharmacological targets. The caspase-1 inhibitor VX-765 reduces IL-1β and IL-18 maturation and limits tissue damage in experimental inflammatory models [137]. Among the compounds investigated as GSDMD inhibitors, necrosulfonamide has shown promising preclinical effects in reducing aneurysm-associated inflammation [138].

Table 1. Therapeutic strategies targeting the NLRP3 inflammasome

Compound	Target	Stage of Development	References
MCC950	Binds to NLRP3 NACHT domain	Preclinical (clinical failure due to hepatotoxicity)	[125,126]
DFV890	Binds to NLRP3 NACHT domain	Phase I/II clinical trial(s)	[127]
Selnoflast (RO7486967)	NLRP3 inhibition	Phase I/II clinical trial(s)	[128]
Dapansutrile (OLT1177)	NLRP3 inhibition	Phase II and III clinical trial(s)[a]	[129-131]
AZD4144	Binds to NLRP3 NACHT domain	Phase II clinical trial(s)[b]	[132]
ML345	Binds to NLRP3 NACHT domain	Preclinical	[133]
Nimbolide	Binds to NLRP3 NACHT domain	Preclinical	[134]
Indole-chalcone hybrids	Binds to NLRP3 NACHT domain	Preclinical	[135]
MF-094	Inhibits USP30 deubiquitinase	Preclinical	[136]
VX-765	Caspase-1 inhibitor	Phase II clinical trial(s)[c]	[137]
Necrosulfonamide	Inhibitor of GSDMD	Preclinical	[138]
Anakinra	IL-1 receptor antagonist	Approved by FDA and EMA	[139]
Canakinumab	IL-1β neutralizing monoclonal antibody	Approved by FDA and EMA	[139]
Rilonacept	IL-1 trap	Approved by FDA and EMA	[139]
Tadekinig alfa	IL-18 binding protein	Phase II and III clinical trial(s)[d]	[140]
siRNA/ASO/CRISPR-based approaches	Gene silencing/editing approaches towards NLRP3	Preclinical	[141]
ANP/BNP (natriuretic peptides)	Natriuretic peptide receptor 1	Preclinical (potential repurposing)	[51,84]

ANP/BNP: atrial and B-type natriuretic peptides; USP30: ubiquitin specific peptidase 30

[a]ClinicalTrials.gov: NCT05658575; [b]ClinicalTrials.gov: NCT07215702; [c]ClinicalTrials.gov: NCT01501383, NCT01048255, NCT00205465; [d]ClinicalTrials.gov: NCT03113760, NCT03512314

Neutralization of inflammasome-derived cytokines remains one of the most effective therapeutic strategies. The IL-1 inhibitors anakinra, canakinumab, and rilonacept are approved for several autoinflammatory and cardiovascular conditions [139]. IL-18 neutralization with tadekinig alfa has also been evaluated in selected immune-mediated diseases [140].

Gene-based approaches, including antisense oligonucleotides, siRNA, and macrophage-targeted CRISPR/Cas9 systems have been used to selectively suppress NLRP3 signalling in experimental models of neuroinflammation, osteoporosis, and inflammatory liver injury [141].
Endogenous pathways also regulate NLRP3 inflammasome activity in different cell types. Atrial and B-type natriuretic peptides (ANP and BNP) inhibit canonical, non-canonical, and alternative inflammasome pathways through the NPR1/cGMP/PKG-I signalling axis. PKG-I-dependent phosphorylation of NLRP3 at S295 reduces inflammasome assembly, caspase-1 activation, and IL-1β maturation [51]. Natriuretic peptides also modulate inflammatory responses in dendritic cells, revealing a functional link between cardiovascular endocrine signalling and immune regulation [84].
Systemic NLRP3 inhibition still presents several limitations, including hepatotoxicity, incomplete control of IL-18 signalling, and limited tissue selectivity [93,126]. In this light, EVs offer a different means of modulating inflammasome activity because they can transfer regulatory cargo and inflammasome-associated molecules between cells or generate soluble mediators that affect NLRP3 signalling [15,142].

Extracellular vesicle biology: a landscape relevant to innate immunity

EVs are membrane-enclosed particles, incapable of autonomous replication that are released by virtually all cell types, involved in intercellular communication. Although initially regarded as cellular debris or particles for cellular waste disposal, EVs are now considered key bioactive carriers that transfer molecular information between cells and across tissues, shaping both physiological and pathological processes [143-145].
The main EV types can be distinguished according to their biogenesis and include ectosomes, exosomes and apoptotic bodies. Apoptotic bodies form during the late stages of programmed cell death from membrane blebbing and apoptopodia fragmentation [146]. Exosomes arise through inward budding of late endosomal membranes, forming intraluminal vesicles within multivesicular bodies (MVB), which subsequently fuse with the plasma membrane to release vesicles extracellularly. This process involves Endosomal Sorting Complexes Required for Transport (ESCRT) proteins, tetraspanins, ceramide-dependent mechanisms, and Rab GTPases [12].
Ectosomes are generated by outward budding of the plasma membrane, driven by cytoskeletal remodelling, calcium-dependent phospholipid redistribution, and membrane curvature regulators [147]. Due to the difficulty in biogenesis-based subtype definition and the lack of universal markers for EV subtypes, the International Society for Extracellular Vesicles (ISEV) discourages the use of biogenesis-related terms unless the EV biogenesis has been deeply characterized [148].
Different EV subtypes are also characterized by distinct size ranges. Small EVs range from approximately 50 to 150 nm (generally <200 nm), whereas large EVs range from 100 to 1,000 nm (generally >200 nm). Apoptotic bodies are more heterogeneous in size, ranging from approximately 500 to 5,000 nm. Because these ranges overlap and strict limits are not universally accepted, size alone is not sufficient for subtype assignment [148]. For these reasons, ISEV recommends the umbrella term “extracellular vesicles” and has established rigorous standards for nomenclature and characterization in the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines [148,149]. In agreement, EVs can be classified using operational criteria based on size, biogenesis (when experimentally supported), and molecular composition (**Table 2**).
EVs carry proteins, lipids, messenger RNAs (mRNA), microRNAs (miRNA), long non-coding RNAs (lncRNA), mitochondrial DNA, and metabolites, and their cargo varies according to the cellular origin and its physiological state. EV membranes are enriched in lipids such as ceramide, cholesterol, sphingomyelin, and phosphatidylserine. Interestingly, the lipid composition of the parent cell membrane differs from that of the released EVs. The latter is characterized by lower levels of phosphatidylcholine and diacylglycerol and higher levels of sphingolipids, saturated fatty acids, and cholesterol [143-145,148]. EVs carry different DNAs, such as genomic DNA and mtDNA, each of which is associated with distinct biological functions. For example, EV-carried mtDNA induces microglial activation in experimental models of traumatic brain injury [144,145].
RNA is another major component of EV cargo and includes mRNA, miRNA, lncRNA, piRNA (PIWI-interacting RNA), and circRNA (circular RNA). Among these, miRNAs are the best characterized because of their role in the post-transcriptional regulation of gene expression [150].

Table 2. Classification of EVs according to MISEV guidelines

EV Category (MISEV-aligned)	Operational Definition	Biogenesis (when known)	Typical Cargo	Common Markers (non-exclusive)
Small EVs (sEVs)	<200 nm	May include EVs of heterogeneous origin (endosomal and plasma membrane-derived)	miRNAs, mRNAs, proteins, lipids, mtDNA	CD9, CD63, CD81, TSG101, Alix
Medium/large EVs (m/lEVs)	>200 nm	Often, but not exclusively, associated with plasma membrane budding	Cytosolic proteins, lipids, signalling molecules, DAMPs	Annexin A1, ARF6, integrins
Apoptotic bodies	~500-5000 nm	Released during apoptosis via membrane blebbing	Nuclear fragments, organelles, DNA, histones	Histones, DNA, phosphatidylserine
Umbrella term				
EVs (undefined subtypes)	EV populations not strictly classified due to overlapping features or insufficient characterization	Mixed or undefined	Variable (proteins, RNAs, lipids, inflammasome components)	Depending on source and isolation method

EV classification follows MISEV guidelines. Size-based terms (e.g., small and large EVs) are used as operational definitions and do not imply specific biogenesis pathways. Terms such as “exosomes” or “microvesicles” are avoided unless their origin is experimentally demonstrated.

EVs also carry a wide variety of proteins, including transmembrane and luminal proteins, which contribute to EV targeting and influence the behaviour of recipient cells [151]. Several EV-associated proteins are widely used as molecular markers, although none is specific for a particular EV subtype [148]. These include the luminal proteins TSG101, Alix, HSC70, and HSP90β, as well as the transmembrane tetraspanins CD9, CD63, and CD81, which are also commonly used for affinity-based EV isolation [148,149].
EVs also contain metabolically active enzymes that can modify the extracellular microenvironment [152]. These bioactive cargo components enable EVs to transfer regulatory information, reprogram recipient cells, and modulate immune and metabolic pathways [11,153]. EV release and cargo composition also vary under different physiological and pathological conditions. For example, during the ageing process, senescent cells show increased secretion of EVs which can spread ageing-related signals [154].
In innate immunity, EVs can transport inflammatory mediators and danger signals, including cytokines, PRR ligands, DAMPs, and inflammasome-associated components, thereby influencing immune responses of the microenvironment through paracrine signalling [13,155].
EVs are also being investigated as both biomarkers and therapeutic carriers. Proteomic analysis of cerebrospinal fluid (CSF)-derived EVs has identified brain-derived proteins, including amyloid precursor protein and prion protein, supporting the concept of CSF-derived EVs as a liquid biopsy of CNS pathology

[156]. Proteomic profiling of CSF-derived EVs from patients with Alzheimer's disease identified enrichment of immune-related proteins, including complement component 1q (C1q), which is involved in synaptic pruning and neuroinflammatory responses [157]. These findings further support the idea that EVs may serve as biomarkers and also support their role in immune regulation in Alzheimer's disease [157].
In this context, technological advancements in quantitative proteomics, RNA sequencing, and single-vesicle approaches are improving the characterization of EV heterogeneity and helping to uncover their function [158].

Crosstalk between extracellular vesicles and the NLRP3 inflammasome in immune regulation

Increasing evidence indicates that EVs are not passive carriers but active regulators of immune signalling, including the NLRP3 inflammasome pathway [9,159]. They transport bioactive cargo, including enzymes and metabolites, which can modify the extracellular environment and, following uptake by recipient cells, alter cellular activity [160-162]. EVs may also carry molecules that influence inflammasome activation. These effects depend on both the cellular origin of the EVs and the recipient cell [15,159]. For example, EVs derived from human amniotic fluid stem cells induce a tolerogenic reprogramming of conventional dendritic cells and attenuate autoimmune neuroinflammation *in vivo* [163].
Inflammasome activation can also alter EV release and cargo composition, generating vesicles that transfer pro-inflammatory or pyroptotic mediators to recipient cells [15,164]. This reciprocal relationship allows EVs to regulate NLRP3 activity, while inflammasome activation itself influences EV cargo and release.

EV-mediated inhibition of NLRP3 activation

EVs derived from immune cells or stem cell populations can inhibit NLRP3-driven inflammation by reducing caspase-1 activation, IL-1β maturation, or pyroptosis [159]. This inhibitory activity has been associated with specific EV cargo, including anti-inflammatory microRNAs, proteins involved in mitochondrial homeostasis, and metabolically active enzymes [15,165] (**Table 3**).
EVs released by macrophages with an activated NLRP3 inflammasome carry a distinct RNA cargo and IFN-β, which induces an IFN response in surrounding macrophages and dampens subsequent NLRP3 inflammasome activation thus exerting a negative feedback effect on neighbouring cells [18]. In a model of bacterial endophthalmitis, EV-associated miR-223-3p has been implicated in the regulation of NLRP3 expression, with increased miR-223-3p levels correlating with reduced NLRP3 expression in vitreous samples from affected patients [166].
Several studies have examined the effects of stem cell-derived EVs, particularly those from mesenchymal stem cells (MSCs), on NLRP3 activation in different tissues. In renal ischaemia/reperfusion injury, MSC-EVs reduce apoptosis and inflammasome activation by delivering miR-223-3p, which directly targets NLRP3 and promotes mitophagy [167]. In cisplatin-induced acute kidney injury, MSC-EVs downregulate HSP70 and NLRP3, with HSP70 identified as a positive regulator of NLRP3 activation [168]. In pulmonary silicosis, EVs derived from 3D-cultured human umbilical cord MSCs and enriched in miR-223-3p counteract circPWWP2A-mediated sequestration of miR-223-3p, reducing NLRP3 activation, macrophage-driven inflammation, and fibrotic remodelling [169].
In pancreatic islets exposed to inflammatory stress, optimized 3D MSC-EVs promote M2 macrophage polarization and reduce IL-1β, caspase-1, and NLRP3 expression, with preservation of β-cell identity [170]. These protective effects were associated with a distinctive miRNA cargo characterized by low levels of miR-127-3p, a promoter of M1 polarization, and high levels of miR-34a-5p and miR-146a-5p, involved in polarization towards the M2 phenotype.
Human MSC-derived small EVs (sEVs) enriched in miR-22-3p reduce microglial activation and inhibit retinal NLRP3/caspase-1 signalling, with improved vascular integrity in experimental diabetic retinopathy [171].
In experimental models of osteoarthritis, human umbilical cord MSC-derived EVs (hUC-MSC-EVs) suppress NLRP3 inflammasome activation through distinct miRNA-mediated mechanisms. hUC-MSC-EV-derived miR-223 directly targets NLRP3, thereby inhibiting inflammasome activation and chondrocyte pyroptosis

[172], whereas miR-1208 targets methyltransferase-like 3 (METTL3), reducing N6-methyladenosine (m6A) modification of NLRP3 mRNA and the associated inflammatory response in macrophages [173].

Table 3. EV-mediated inhibition of NLRP3 activation

EV Source	Cargo	Target Cells	Effect on NLRP3 Inflammasome	References
Activated macrophages	IFN-β, regulatory RNAs	Macrophages	Reduction of NLRP3 activation	[18]
Not identified	miR-223-3p	Retinal cells	Reduction of NLRP3 expression	[166]
Mouse bone marrow-MSCs	miR-223-3p	Renal cells	Reduction of NLRP3 expression and activation; via mitophagy enhancement	[167]
Human bone marrow-MSCs	Not identified	Renal cells	Suppression of NLRP3 inflammasome activation via HSP70 downregulation	[168]
Human umbilical cord-derived MSCs	miR-223-3p	Macrophages	Suppression of NLRP3 via circPWWP2A/miR-223 axis	[169]
Human umbilical cord blood-derived MSCs	miR-34a-5p; miR-146a-5p enriched	Islets-resident macrophages / pancreatic islets	Reduction of NLRP3 and caspase-1 expression, promotion of M2 polarization	[170]
Human umbilical cord blood-derived MSCs	miR-22-3p	Microglia	Suppression of NLRP3 via miR-22-3p-mediated targeting of NLRP3	[171]
Human umbilical cord MSCs	miR-223	Chondrocytes	Suppression of NLRP3 via direct miR-223 targeting; inhibition of pyroptosis	[172]
Human umbilical cord MSCs	miR-1208	Macrophages	Suppression of NLRP3 via direct miR-1208/METTL3-mediated reduction of NLRP3 mRNA m6A modification	[173]
Human amniotic fluid-derived stem cells	ATP-to-adenosine (CD39/CD73)	Monocytes (THP-1)	Suppression of NLRP3 activation via adenosine A2A receptor signalling	[178]
Human bone marrow-derived MSCs	Anti-inflammatory cytokines	Microglia	Suppression of NLRP3 activation	[175]

EV Source	Cargo	Target Cells	Effect on NLRP3 Inflammasome	References
Human umbilical cord-derived MSCs	Not identified	Microglia	Inhibition of NLRP3 activation through enhanced ubiquitination	[180]
Human MSCs	miRNAs	Microglia	Inhibition of NLRP3 activation and NLRP3-p38/MAPK signalling	[181]
Astrocytes	GJA1-20k	Neurons	Suppression of NLRP3-associated pyroptosis via Pink1-dependent mitophagy	[182]
hiPSC-derived neural stem cells	Not identified	Microglia; astrocytes	Suppression of NLRP3 activation and associated neuroinflammatory signalling	[177]
Engineered EVs (SHP2-enriched)	SHP2	Neurons	Induction of mitophagy, indirectly reducing NLRP3 activation	[183]
GMP-manufactured neural stem cells	Immunomodulatory mediators (CD73, miR-21)	Monocytes/microglia	Suppression of caspase-1 activation and inflammatory responses	[179]
M2 microglia	miR-135a-5p	Neurons	Inhibition of NLRP3 activation via miR-135a-5p/TXNIP axis	[174]

MSC: mesenchymal stem cells; hiPSC: human-induced pluripotent stem cell; SHP2: Src homology region 2 domain-containing phosphatase 2; GMP: good manufacturing practice; TXNIP: thioredoxin-interacting protein; METTL3: methyltransferase-like 3; m6A: N6-methyladenosine.

Microglia-derived EVs enriched in miR-135a-5p downregulate neuronal thioredoxin-interacting protein (TXNIP), a positive regulator of NLRP3, reducing inflammasome assembly and ischaemic brain injury [174]. Intranasal administration of bone marrow MSC-derived EVs improved motor recovery after cortical stroke and was associated with reduced neuroinflammation [175].

Intranasally delivered EVs from human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) reduce inflammasome activation and neuroinflammation in Alzheimer's disease and ageing models [176,177]. In a model of peripheral inflammation-induced cognitive impairment, these EVs reduced neuroinflammatory markers, attenuated NLRP3 inflammasome activation, and restored hippocampal neurogenesis [176].

EVs can also affect inflammasome activity through uptake-independent mechanisms. Stem cell-derived EVs can regulate NLRP3 activation through the production of soluble mediators. Human amniotic fluid stem cell-derived EVs autonomously generate ATP through the pay-off phase of glycolysis. ATP is then converted to adenosine by the ectonucleotidases CD39 and CD73, leading to A2A adenosine receptor activation and inhibition of LPS/ATP-induced NLRP3/caspase-1 activation in THP-1 cells [178].

Similarly, Good Manufacturing Practice (GMP)-manufactured human neural stem cell-derived EVs reduced nitric oxide production and inducible nitric oxide synthase expression in LPS-stimulated microglia and inhibited caspase-1 activation in monocytic models through both uptake-dependent and uptake-independent

mechanisms [179]. These findings suggest that, in addition to delivering molecular cargo, EVs can act as autonomous metabolic units capable of promoting immunomodulation.
Other mechanisms have also been proposed. EVs of different cellular origins, including MSC-derived, astrocyte-derived, and engineered EVs, have been shown to suppress NLRP3 activation in several experimental models of neuroinflammation, including ischaemic stroke, neonatal hypoxic-ischaemic injury, traumatic brain injury, Alzheimer's disease, and spinal inflammation associated with interstitial cystitis [175,180-184]. These effects are associated with reduced expression of NLRP3, ASC, cleaved caspase-1, IL-1β, and IL-18, together with improved functional outcomes in several models. Reported mechanisms include inhibition of TLR4/NF-κB-dependent priming [184], modulation of NLRP3/p38 MAPK signalling [181], promotion of NLRP3 ubiquitination and degradation [180], and induction of mitophagy [183].
These studies show that EV-mediated inhibition of NLRP3 can occur through different mechanisms depending on EV origin and target cell.

EV-mediated activation of the NLRP3 inflammasome

EVs released by activated, stressed, or pathological cells can instead propagate and amplify inflammasome signalling in recipient cells (**Table 4**).

Table 4. EV-mediated activation of the NLRP3 inflammasome

EV Source	Cargo	Target Cells	Effect on NLRP3 Inflammasome	References
IL-1β-stimulated chondrocytes	miR-449a-5p	Macrophages	Enhanced NLRP3 activation	[185]
COVID-19 patient plasma	Not identified	Macrophages	Enhanced IL-1β	[186]
Hepatocytes (microcystin-LR exposure)	miR-328-3p	Macrophages	Enhanced NLRP3 activation via PP2A-B56δ upregulation	[187]
Lung (sepsis)	miR-128-3p	Macrophages	Enhanced NLRP3 expression via Rab20 targeting	[188]
Choroid plexus epithelial cell	miR-155-5p	Neurons	Enhanced NLRP3 activation via Rheb suppression	[189]
Endothelial cell	Not identified	Trophoblasts	Enhanced NLRP3 activation via platelet-derived ATP signalling	[190]
Advanced-stage prostate cancer cells	Not identified	Prostate epithelial cells	Enhanced NLRP3, caspase-1, and IL-1β activation via ERK1/2-dependent lysosomal destabilization and cathepsin B activation	[191]
Lung tumour cells	TRIM59	Macrophages	Enhanced NLRP3 activation via ABHD5 degradation	[192]

Rheb: Ras homolog enriched in brain; PP2A: serine/threonine-protein phosphatase 2A; TRIM59: tripartite motif-containing protein 59; ABHD5: α/β-hydrolase domain-containing protein 5.

In experimental osteoarthritis, exosome-like vesicles released by damaged chondrocytes can activate synovial macrophages and increase IL-1β production [185]. EVs isolated from patients with severe COVID-19 have also been shown to induce lung inflammation, promote macrophage M1 polarization, and enhance the production of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α [186].
Pathological EVs also modulate inflammasome signalling through alterations in their miRNA cargo. Hepatocyte-derived EVs released after microcystin-LR exposure show reduced transfer of miR-328-3p, with increased PP2A-B56δ activity and phosphorylation-dependent NLRP3 activation [187]. In experimental sepsis, lung-derived EVs transfer miRNAs, including miR-128-3p, to macrophages, promoting M1 polarization and increasing TNF-α and IL-6 production through Rab20 targeting [188].
In the CNS, choroid plexus epithelial cell-derived EVs enriched in miR-155-5p exacerbate ischaemic brain injury by suppressing Ras homolog enriched in brain (Rheb) and promoting autophagy-dependent NLRP3 inflammasome activation in neurons [189]. In preeclampsia, EVs promote platelet activation and ATP release, leading to purinergic inflammasome activation in trophoblasts and contributing to sterile placental inflammation [190].
Tumour-derived EVs can also activate inflammasome signalling and IL-1β production in cells of the tumour microenvironment, reinforcing inflammatory pathways that support tumour growth, immune evasion, and resistance to chemotherapy [122,191]. In lung carcinoma, EV-mediated transfer of the ubiquitin ligase TRIM59 to macrophages promotes ABHD5 degradation and metabolic reprogramming, followed by NLRP3 activation, caspase-1 processing, and IL-1β secretion [192].
EVs released under pathological conditions can therefore transfer NLRP3-activating signals to other cells, in the same or distal tissues, extending inflammatory responses beyond the site of primary injury and potentially contributing to disease progression.

EV-mediated spreading of NLRP3 inflammasome signalling

It has been increasingly reported that inflammasome activation can promote the release of EVs enriched in inflammasome components and pro-inflammatory mediators (**Table 5**).
Macrophages undergoing NLRP3 activation release exosome-like vesicles containing NLRP3, ASC, caspase-1, and mature IL-1β, which can activate NF-κB signalling and inflammatory gene expression in recipient macrophages [14]. Beyond cargo transfer, the magnitude and propagation of EV-mediated inflammasome signalling are also influenced by cellular mechanisms regulating EV secretion. One key mechanism involves the fate of MVBs. Under physiological conditions, MVBs can undergo lysosomal degradation or fuse with the plasma membrane to release exosomes. Under pathological conditions, lysosomal dysfunction favours the latter pathway and increases the release of EVs enriched in inflammasome-associated cargo [193,194].
In hyperhomocysteinaemic conditions, NADPH oxidase-derived ROS are involved in the generation of MVBs enriched in inflammatory cytokines and induce NLRP3 activation in podocytes [193]. Moreover, the deficiency of lysosomal acid ceramidase in endothelial cells controls the release of NLRP3 inflammasome products via EVs during hyperglycaemia [194]. Furthermore, endothelial cells respond to 7-ketocholesterol exposure by reducing lysosome-MVB fusion and increasing the secretion of EVs containing IL-1β [195]. Lysosomal function can therefore influence whether inflammasome components are degraded or released through EVs.
All these findings strongly suggest that inflammasome activation can both promote EV release and alter their molecular cargo [13]. Indeed, neutrophil-derived EVs carrying active caspase-1 transfer inflammasome signals to neighbouring neutrophils and epithelial cells, inducing caspase-1 activation and IL-1β release in cystic fibrosis models [196].
In experimental sickle cell disease, platelet inflammasome activation promotes the release of EVs containing caspase-1 and IL-1β. These EVs bind neutrophils, promoting platelet-neutrophil aggregation and lung vaso-occlusion [197].
EVs can also initiate inflammasome activation in recipient cells by delivering DAMPs, including ATP, oxidized mitochondrial DNA, misfolded proteins, and bioactive lipids that function as canonical triggers of NLRP3 assembly [198]. Host-derived EVs can bind circulating LPS and deliver it to the cytosol of recipient cells through a CD14-dependent mechanism, activating the non-canonical inflammasome pathway and GSDMD-

mediated pyroptosis [199]. Interestingly, microbiota-associated human amniotic fluid-derived EVs (hAF-EVs) can produce different effects according to the timing of exposure. In THP-1 cells, hAF-EVs induce caspase-1 activation but reduce subsequent LPS/ATP-induced inflammasome activation [200].

Table 5. EV-mediated spreading of NLRP3 inflammasome signalling

EV Source	Cargo	Target Cells	Effect	References
Inflammasome-activated macrophages	NLRP3, ASC, IL-1β, caspase-1	Macrophages	NF-κB-dependent NLRP3 priming and enhanced subsequent activation	[14]
Neutrophils	Active caspase-1	Neutrophils / epithelial cells	Feed-forward NLRP3 inflammasome activation	[196]
Platelets	Caspase-1, IL-1β	Neutrophils	Induction of platelet-neutrophil aggregation and pulmonary vaso-occlusion	[197]
Plasma-derived	LPS	Macrophages	Non-canonical inflammasome activation and pyroptosis	[199]
Human amniotic fluid	EV-associated LPS	Monocytes	Bimodal regulation of inflammasome activation	[200]
LPS-stimulated macrophages	HMGB1	Hepatocytes	NLRP3 inflammasome activation and pyroptosis	[201]
Pyroptotic cells	Gasdermin D pore structures	Multiple cell types	Induction of pyroptosis	[164]
NLRP3-activated macrophages	NLRP3, IL-1β inflammatory proteins	Macrophages / endothelial cells	Amplification of inflammatory responses	[15]

HMGB: high mobility group box 1.

In addition to soluble danger signals and microbial products, EVs can also transport endogenous alarmins. Macrophage-derived EVs deliver high mobility group box protein 1 (HMGB1) to hepatocytes through transferrin-mediated endocytosis, inducing NLRP3 activation and pyroptosis [201]. Mitochondrial DNA represents another EV-associated danger signal. Oxidized mtDNA activates NLRP3 [62,202], and mtDNA packaged into EVs can retain biological activity after transfer to recipient cells [203].

Beyond DAMPs, EVs can also disseminate functional components of the pyroptotic machinery. EVs released during pyroptosis can carry GSDMD pore structures, whose transfer to recipient cells induces membrane permeabilization and pyroptotic cell death [164].

Proteomic analysis of EVs released after NLRP3 activation identified a distinct EV population termed “infosomes”. These vesicles are selectively enriched in inflammatory proteins, are released in an NLRP3-dependent manner, and induce inflammatory gene expression programmes in both macrophages and endothelial cells [15].

Inflammasome activation also generates extracellular inflammatory structures that are distinct from EVs. ASC specks released from pyroptotic cells persist extracellularly and can be internalized by neighbouring phagocytes, where they promote further inflammasome activation [204]. Although ASC specks are not EVs, both mechanisms can transfer inflammasome-associated signals between cells.

The relationship between EVs and NLRP3 is therefore reciprocal. EVs can modify inflammasome activity in recipient cells, whereas NLRP3 activation can alter EV biogenesis and cargo composition, influencing their inflammatory potential.

Discussion

The studies reviewed here show that NLRP3 regulation can be guided by long-range signalling. EV-mediated communication can either increase or reduce inflammasome activity in recipient cells, depending on their origin and cargo. This property makes EVs relevant to the development of strategies aimed at modulating NLRP3 activity.

EV surface molecules, including adhesion proteins, integrins, tetraspanins, and specific glycan patterns, influence their uptake by immune and stromal cells [11]. This differs from the broad systemic distribution of small-molecule NLRP3 inhibitors and may allow more selective delivery to specific cell populations.

EV cargo includes miRNAs, lncRNAs, proteins, lipids, and metabolites that can affect different steps of NLRP3 signalling. EV-associated miRNAs, for example, can regulate NLRP3 expression, inflammasome assembly, and upstream priming signals [11,13].

A critical step towards clinical translation is the development of GMP-compatible manufacturing and standardized quality-control procedures. Reproducible production, clearly defined release criteria, and reliable potency assays are required to ensure the consistency and biological activity of EV-based products before their use in clinical applications [205,206].

EV engineering offers additional possibilities for NLRP3-directed delivery. Current approaches include (i) endogenous loading by engineering of producer cells, and/or (ii) exogenous post-isolation loading and surface functionalization. These strategies are increasingly examined as routes for the delivery of NLRP3-suppressive nucleic acids, proteins, or small molecules preferentially to disease-relevant cells [207,208].

Several issues still limit the clinical development of EV-based approaches to NLRP3 modulation.

The first challenge is the identification of the molecular drivers of EV-mediated immunomodulation. EV preparations are intrinsically heterogeneous and may contain multiple active cargo components, including miRNAs, proteins, lipids, and metabolites, which may act individually or in combination. Determining which components are causally responsible for inflammasome inhibition or activation will be important for rational therapeutic design [148].

Another challenge concerns the accurate assessment of EV biodistribution and targeting. EV tracking is method-dependent and can be confounded by dye transfer or dissociation, and EVs may accumulate preferentially in reticuloendothelial organs. Standardized tracking methods and quantitative measures of target engagement are therefore needed [209,210].

The mechanisms responsible for EV activity also require further clarification. In many EV-based systems, it remains unclear whether their effects are primarily mediated by intracellular cargo delivery following cellular uptake, receptor-ligand interactions at the cell surface, soluble mediators, or a combination of these mechanisms. This question is particularly relevant to NLRP3 regulation, because EVs may act at different stages of inflammasome signalling, from priming to cytokine secretion and pyroptosis.

Manufacturing represents an additional challenge, particularly regarding batch-to-batch variability and EV heterogeneity. Donor variability, culture conditions, isolation protocols, and storage procedures can significantly affect EV composition and biological function. Standardization efforts, including the MISEV guidelines, provide important reference points, but ensuring comparability across production pipelines remains difficult [148].

Long-term safety also requires careful consideration. It remains unclear whether prolonged EV-mediated suppression of NLRP3 could interfere with physiological inflammasome functions involved in host defence, immune surveillance, or tissue repair. Future studies will need to determine whether NLRP3 activity can be reduced sufficiently to control chronic inflammation without compromising these protective functions.

Conclusions

Current evidence supports a reciprocal relationship between NLRP3 inflammasome activity and EV-mediated communication. EVs can deliver cargo that increases or reduces inflammasome activity in recipient cells, while NLRP3 activation can modify EV release and cargo composition. This relationship extends inflammasome regulation beyond the individual cell and may contribute to the propagation or control of inflammatory responses.

Systemic NLRP3 inhibition is limited by toxicity and lack of tissue selectivity. In this scenario, EV-based approaches may provide alternative means of modulating NLRP3 activity in selected cell populations, but important questions concerning EV cargo, targeting, manufacturing, mechanisms of action, and long-term safety remain to be resolved.

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Author Contributions

IB: Conceptualization; writing-original draft; writing-review and editing; **DC:** critical revision for important intellectual content and editing; **MG:** critical revision for important intellectual content and editing; **LM**: Conceptualization; writing-original draft; writing-review and editing; **RR**: critical revision for important intellectual content and editing; **VNT**: critical revision for important intellectual content and editing.
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Competing interests

The Authors declare that they have no conflict of interest to disclose.

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