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INVITED REVIEW

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Lipids, inflammasomes, metabolism, and disease

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Abstract

Inflammasomes are multi-protein complexes that regulate the cleavage of cysteine protease caspase-1, secretion of inflammatory cytokines, and induction of inflammatory cell death, pyroptosis. Several members of the nod-like receptor family assemble inflammasome in response to specific ligands. An exception to this is the NLRP3 inflammasome which is activated by structurally diverse entities. Recent studies have suggested that NLRP3 might be a sensor of cellular homeostasis, and any perturbation in distinct metabolic pathways results in the activation of this inflammasome. Lipid metabolism is exceedingly important in maintaining cellular homeostasis, and it is recognized that cells and tissues undergo extensive lipid remodeling during activation and disease. Some lipids are involved in instigating chronic inflammatory diseases, and new studies have highlighted critical upstream roles for lipids, particularly cholesterol, in regulating inflammasome activation implying key functions for inflammasomes in diseases with defective lipid metabolism. The focus of this review is to highlight how lipids regulate inflammasome activation and how this leads to the progression of inflammatory diseases. The key roles of cholesterol metabolism in the activation of inflammasomes have been comprehensively discussed. Besides, the roles of oxysterols, fatty acids, phospholipids, and lipid second messengers are also summarized in the context of inflammasomes. The overriding theme is that lipid metabolism has numerous but complex functions in inflammasome activation. A detailed understanding of this area will help us develop therapeutic interventions for diseases where dysregulated lipid metabolism is the underlying cause.

KEYWORDS

cholesterol, homeostasis, inflammasome, lipids, metabolism, NLRP3

1 | INTRODUCTION

The inflammasome is a multi-protein structure consisting of a sensor protein complexed to effector caspase-1 through an adapter protein ASC (apoptosis-associated speck-like protein containing a caspase activation and recruitment domain [CARD]).[1-3] The sensor protein, mostly a member of the nod-like receptor (NLR) family, is a tripartite protein consisting of a central NACHT domain, an amino-terminal pyrin domain (PYD), and a carboxy-terminal leucine-rich repeat domain (LRR).[4] The ASC protein (encoded by *PYCARD* gene) consists of an amino-terminal PYD domain and a carboxy-terminal CARD. By contrast, caspase-1 has an amino-terminal CARD domain, a central large catalytic domain, and a carboxy-terminal small catalytic subunit domain.[5-7] The LRR domain of the NLR protein is thought to inhibit

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auto-activation of the inflammasome. During steady state, the LRR domain folds back onto the NACHT domain of the sensor molecule which displays an ATPase function deemed fundamental for NLRP3 activity. Upon sensing and activation by apt stimuli, the sensor protein, for example, NLRP3, oligomerizes and further recruits ASC through homotypic interactions between the PYD domains of the two proteins. This results in helical ASC filament formation which assembles into a large ASC "speck".[8,9] Upon the formation of ASC speck, caspase-1 is recruited through homotypic CARD-CARD interactions resulting in proximity-induced caspase-1 self-cleavage and activation through cleavage at the p20-p10 linker region.[10] Active caspase-1 results in proteolytic processing of inactive precursor forms of cytokines IL-1β and IL-18 to their biologically active forms. Caspase-1 also cleaves gasdermin D to its active N-terminal fragment which induces an inflammatory form of cell death known as pyroptosis.[11-14] Besides these core molecules that make up the NLRP3 inflammasome, caspase-8 and FADD are also key to NLRP3 activation and participate in priming and activation of the NLRP3 inflammasome.[15,16] Similarly, a serine-threonine kinase involved in mitosis, NEK7, is also required for NLRP3 activation.[17,18]

The activating mechanism of the NLRP3 inflammasome has been puzzling since its initial discovery. A wide range of chemically distinct stimuli can activate the NLRP3 inflammasome,[19,20] including bacterial and viral pathogen-associated molecular patterns (PAMPs), and endogenous danger-associated molecular patterns (DAMPs) released from damaged or dying cells.[21-24] The latter stimuli form the basis for eliciting sterile inflammation.[22,25,26] Activation of NLRP3 is recognized to occur in two steps. The priming step upregulates NLRP3 and pro-IL-1β. The activation step is mediated by PAMPs and DAMPs and involves the downstream generation of mitochondrial reactive oxygen species (mtROS), phagolysosomal damage, or potassium efflux.[27-30] NLRP3 also assembles a non-canonical inflammasome involving caspase-11 which is activated by Gram-negative bacteria.[31-33] Lipopolysaccharide (LPS) from Gram-negative bacteria directly binds caspase-11 in the cytoplasm and prompts non-canonical NLRP3 activation and pyroptosis.[34,35]

Though NLRP3 inflammasome is activated in response to multiple stimuli, assembly of other inflammasomes is specific to their ligands. Upon recognition of bacterial flagellin and certain type III secretion system components, NLRC4 inflammasome is activated.[36-39] The ligand recognition in the NLRC4 inflammasome is executed by upstream NAIP proteins (NLR family of apoptosis inhibitory protein). The mouse genome encodes four different NAIPs (NAIP1, NAIP2, NAIP5, and NAIP6) while only one NAIP is expressed in humans. The assembly of NLRC4 inflammasome differs from NLRP3 inflammasome as the core oligomer is composed of both NLRC4 and NAIP at an average composition of five NLRC4 to two NAIP monomers.[40] Remarkably, ligand specificity is not determined by the NAIP LRR domain but is implemented by helical domains associated with NACHT domain.[40] On the other hand, the absent in melanoma 2 (AIM2) inflammasome is activated in response to the cytoplasmic presence of double-stranded DNA (dsDNA).[41-45] Recognition of DNA by AIM2 is accomplished by electrostatic attraction between the positively charged HIN domain residues of AIM2 and the sugar-phosphate backbone of dsDNA.[46] The footprint of one $AIM2^{HIN}$ on dsDNA is eight to nine base pairs and multiple AIM2 molecules can bind the same DNA simultaneously. Moreover, recognition of DNA by AIM2 is sequence-independent and requires approximately 80 base pairs for optimal inflammasome assembly.[46,47] The activation of the NLRP1 (coded by a specific mouse allele, *Nlrp1b*) inflammasome is most well documented in response to anthrax lethal toxin from *Bacillus anthracis* which can directly cleave and activate this inflammasome.[48] However, NLRP1 is also activated in response to Toxoplasma infection.[49,50] Direct interaction of NLRP1 with a pathogen-associated molecule has not been demonstrated yet raising speculations that NLRP1 might be a general sensor of cellular homeostasis. More recently, inhibition of host cell serine proteases DPP8/DPP9 has been shown to activate all rodent NLRP1 alleles.[51,52] Notably, the sensitivity of NLRP1 to DPP8/9 inhibitor-induced pyroptosis is extremely similar to *Toxoplasma gondii*-induced pyroptosis suggesting that NLRP1 might indeed be sensing a specific pathogen stimuli.[51] The identity of this signal remains unknown. Although adapter protein ASC is critical in oligomerizing most NLRs with effector caspase-1, exceptions to these are the NLRP1 and NLRC4 inflammasomes where the sensor proteins can potentially activate caspase-1 without ASC recruitment.[53,54] While the precise mechanistic details of the assembly remain obscure, the involvement of ASC in these inflammasomes elevates caspase-1 activity and downstream IL-1β and IL-18 production.

Lipids are fundamental in shaping cellular architecture and play pivotal roles in diverse cellular processes.[55-57] In particular, they are key structural elements providing rigidity and permeability to biological membranes, the most notable of which is the plasma membrane (PM). Lipids also contribute to defining the distinct characteristics and functional nature of various organelles by regulating the trafficking of molecules between their membranes.[58,59] Additionally, they trigger defined biological processes in their roles as second messengers and maintain reserve energy stores in the form of lipid droplets.[57]

Lipid metabolism is known to directly influence inflammatory processes.[57,60,61] Thus, dysregulation in lipid metabolism underlies the etiology of several diseases including cardiovascular disease and diabetes, which in several settings are instigated by chronic inflammation. This has boosted interest in how lipid metabolism is reconfigured during disease and how it might shape immune responses.[62,63] Notably, lipid efflux and TLR signaling are rigidly associated. Homeostatic responses to cellular lipid loading are mediated by the liver X receptors (LXRs), which, by their transcriptional activity, contribute to lipid transport and disposal. The natural ligands for LXRs, oxysterols, are generated as a result of macrophage uptake of oxidized low-density lipoproteins (LDL).[64,65] Upon activation, LXRs code for genes involved in lipid efflux including ATP-binding cassette (ABC) transporters A1 (ABCA1), ABCG1, and phospholipid transfer protein.[66] While ABCG1 is involved in cholesterol efflux to high-density lipoproteins, ABCA1 has a broader role and promotes efflux of both cholesterol and phospholipids to apolipoproteins. In

macrophages lacking *Abca1*, elevated cholesterol accumulation is accompanied by enhanced TLR-mediated proinflammatory signaling. By contrast, TLR3- and TLR4-mediated IRF3 signaling restrain LXR-induced ABCA1 expression thereby preventing cholesterol efflux and thus promoting atherogenic activity.[67,68] Other proinflammatory mediators are also involved in the downregulation of ABCA1. Though TNF-α and IL-6 are one of the earliest cytokines secreted upon TLR stimulation, they, however, upregulate macrophage ABCA1 expression via the NF-κB pathway.[69-71] It is worth mentioning that according to the emerging view, NF-κB may be both involved in the progression and resolution of inflammatory lesions.[72] In contrast to the above, LXR ligands inhibit the expression of inflammatory mediators (inducible nitric oxide synthase and cyclooxygenase 2) upon LPS exposure in vitro and decrease inflammation in mouse models of contact dermatitis, and inflammatory gene expression in the aortas of atherosclerotic mice.[73] These studies highlight the intricate relationship between inflammatory signaling and lipid efflux which holds critical implications in the context of a variety of lipid-associated disorders including cardiovascular diseases.

In addition to their roles in inflammatory diseases, lipids, and in particular cholesterol-rich domains known as membrane rafts, are used as entry points to the cells by pathogens. Once inside, pathogens rely on host cholesterol machinery to survive and proliferate by either upregulating cholesterol biosynthesis or hijacking the transport of existing cellular cholesterol to their own intracellular vacuoles.[74-78] By contrast, effective immunity to pathogens also relies heavily on lipids. Lipids regulate immune signaling to pathogens through conserved pattern recognition receptors and their adapter molecules.[67,79] In this context, it has been suggested that the host LPS-binding protein (LBP), which recognizes lipid A moiety in the bacterial LPS, and phospholipid transfer protein both share significant homology and have been proposed to belong to a putative common gene family of lipid-binding proteins.[80] Additionally, LPS and lipids share uptake and efflux pathways. Cellular uptake of LPS is promoted by scavenger receptor B1 while ABCA1 can efflux LPS along with phospholipids and free cholesterol.[81,82] Together, these studies implicate lipids in driving homeostatic immune processes—in host defense and inflammatory diseases.

The focus of this review is how lipids contribute to inflammasome activation, and how this activation drives the development of chronic inflammatory diseases. Since cholesterol and lipid metabolism are primarily involved in the regulation of the NLRP3 inflammasome, thus, the discussion is mainly focused on this inflammasome and other inflammasome types are discussed when relevant and studied in that context.

2 | NLRP3 INFLAMMASOME IS REGULATED BY NPC1 AND ENDOPLASMIC RETICULUM CHOLESTEROL LEVELS

Cellular cholesterol homeostasis is maintained by cholesterol uptake, biosynthesis, and efflux programs, which are orchestrated by distinct cholesterol sensors and antagonistic transcription factors.[59,83,84] Nucleated cells mainly obtain cholesterol from exogenous dietary sources in the form of LDLs by the clathrin-mediated endocytosis of LDL-receptor.[85,86] Once LDL reaches the late endosome/lysosome compartment, acid lipases hydrolyze the cholesterol ester within the LDL core.[87] Subsequently, free cholesterol is effluxed out of the acidic organelle through a lysosome-localized transmembrane cholesterol transporter, Niemann-Pick C1 (NPC1).[88-91] Subsequently, cholesterol is distributed heterogeneously to distinct membranes depending on their individual requirements.[92,93]

Cholesterol can also be synthesized de novo in the endoplasmic reticulum (ER) by the coordinated actions of transcription factor sterol regulatory element-binding protein 2 (SREBP2), adapter proteins, and proteases.[91] Under steady-state conditions, cholesterol binds to the sterol sensing domain of the escort protein SREBP cleavage-activating protein (SCAP) thereby keeping it tethered to insulin-induced gene (INSIG).[94] This interaction keeps the SREBP2-SCAP complex inactivate and sequestered in the ER. However, when cellular cholesterol levels drop, the sterol-free form of SCAP dissociates from INSIG and chaperones SREBP2 to the *cis*-Golgi for processing (Figure 1).[94] Here, site-1 protease (S1P) and site-2 protease (S2P) sequentially cleave SREBP2 to generate the active N-terminal fragment (Figure 1).[94] Active SREBP2 then translocates to the nucleus to transcribe genes involved in cholesterol uptake and biosynthesis, including LDL-R and HMG-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway (Figure 1).[84,91] By contrast, cholesterol clearance is mainly driven by the transcription factor, LXRs, which mediate cholesterol efflux through upregulation of ABCA1 and ABCG1.[94]

Elevated cholesterol is a major trigger in cardiovascular disease. One previous report documented that Apo-E-deficient mice on high-cholesterol diet display crystal-like structures, which stained positive with cholesterol stain filipin, in early atherosclerotic lesions.[95] Notably, cholesterol crystals, a hallmark of atherosclerotic lesions, were historically associated with only mature lesions and therefore ignored as the primary inflammatory stimuli in atherosclerosis.[95-98] By employing novel microscopy approaches, the investigators found that the appearance of cholesterol crystals coincides with the earliest recruitment of inflammatory cells suggesting cholesterol crystals as the instigating stimuli in atherosclerosis.[95] Exposure of LPS-primed mouse bone marrow-derived macrophages (BMDMs) to cholesterol crystals in vitro resulted in increased NLRP3 inflammasome activation and IL-1β release in wildtype (WT), but not in *Nlrp3*$^{-/-}$, macrophages.[95] Mechanistically, cholesterol crystals induced phagolysosomal damage resulting in the leakage of lysosomal contents including cathepsins into the cytoplasm which activated the NLRP3 sensor protein.[95,99] Accordingly, cells deficient in cathepsin B revealed reduced caspase-1 cleavage in response to cholesterol crystals. These studies, therefore, proposed phagolysosomal damage as a key mechanism of NLRP3 activation. Indeed, other crystalline substances also activate NLRP3 inflammasome,[29,100] thereby emphasizing the competence of particulate

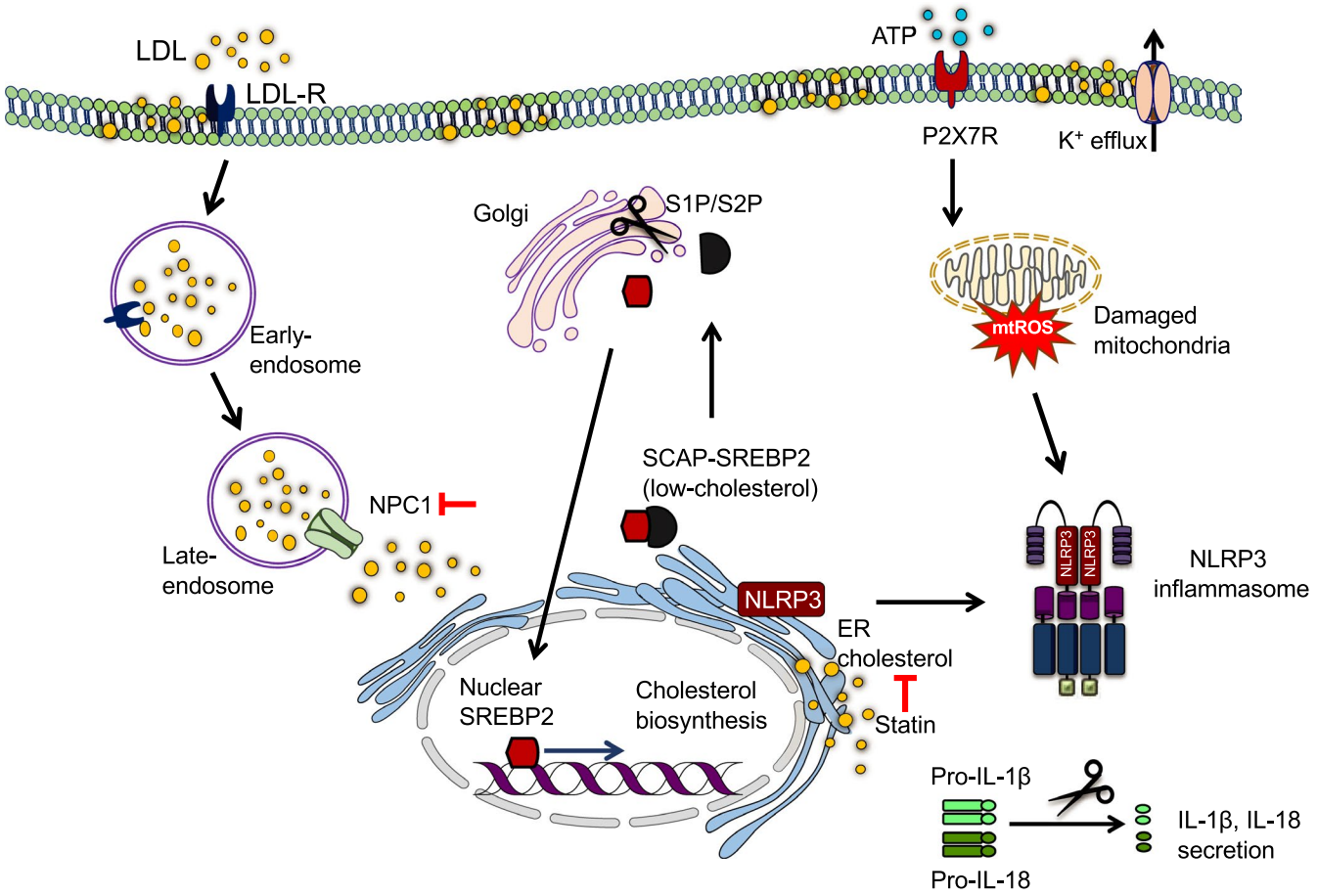


FIGURE 1 NLRP3 inflammasome is regulated by NPC1 and ER cholesterol levels. Cholesterol is obtained by exogenous uptake of low-density lipoproteins (LDL) by the endocytosis of LDL-R. Upon reaching the late endosome, cholesterol esters are hydrolyzed, and free cholesterol is effluxed out of the lysosome compartment by NPC1. Subsequently, cholesterol is heterogeneously distributed to distinct cellular compartments. Under low-cholesterol conditions, SCAP chaperones SREBP2 to the Golgi for sequential processing by S1P and S2P to generate the cleaved nuclear SREBP2. Active SREBP2 fragment translocates to the nucleus where it transcribes genes involved in cholesterol biosynthesis and uptake. NPC1-blockade by pharmacological or genetic approaches results in lysosomal cholesterol accumulation resulting in decreased ER cholesterol pool. Treatment with statins can also acutely deplete ER cholesterol pool. Disruption of ER cholesterol levels dampens activation of the inflammasome in response to NLRP3 stimuli. LDL-R, low-density lipoprotein receptor; NPC1, Niemann-Pick type C1; ER, endoplasmic reticulum; SREBP2, sterol regulatory element-binding protein 2; SCAP, SREBP cleavage-activating protein; S1P/S2P, site-1 protease/site-2 protease

stimuli to activate NLRP3-dependent functions solely due to their physical properties.

Besides a pivotal role for cholesterol crystals, homeostatic cholesterol metabolism and trafficking have been recently recognized to regulate immune functions.[61] Cholesterol trafficking impacts dendritic cell (DC) function, antibody responses, and outcome of pathogen invasion.[101-103] Moreover, perturbations in lipid metabolism underlie diverse human pathologies including cardiovascular disease and Alzheimer's.[83] Finally, genetic defects in lipid and cholesterol trafficking lead to Niemann-Pick and Tangier disease.[104,105] Therefore, improved understanding of how cholesterol regulates immune responses might help develop novel therapies for a range of human diseases. However, whether cholesterol trafficking influenced inflammasome activation was not known. A principal step in the cholesterol trafficking pathway is the lysosomal efflux of cholesterol through NPC1.[91] Consequently, NPC1 blockade results in lysosomal cholesterol accumulation (Figure 1).[106] In agreement, pharmacological inhibition of NPC1 in mouse BMDMs resulted in cholesterol accumulation, but intriguingly coincided with reduced caspase-1 activity in response to NLRP3 stimuli.[106] Genetic deletion of *Npc1* mimics the above results revealing blunt IL-1β secretion; however, TNF-α production and release are not restricted indicating no effect of the loss of NPC1 function on the NLRP3 priming step. Moreover, *Npc1* deletion does not hinder activation of the NLRC4 and AIM2 inflammasomes[106] proposing that NPC1-dependent cholesterol trafficking pathway exclusively affects the NLRP3 inflammasome.

NPC1 functions to transport cholesterol out of lysosomes to distinct cellular organelles. Therefore, together with the above studies, this implies a role for postlysosomal cholesterol pool in NLRP3 inflammasome activation. By employing pharmacological approaches in WT cells to independently deplete PM and ER cholesterol pools, which

are contracted in cells lacking *Npc1*,[107,108] the authors next delineated the contributions of the two organelles in inflammasome activation. While PM cholesterol did not influence NLRP3 activation, depletion of ER cholesterol blunted inflammasome activity (Figure 1).[106] Notably, cholesterol levels in the ER are tightly regulated but can be acutely manipulated. By exploiting established approaches which involved culturing cells in lipoprotein-deficient media, the investigators forced cells to rely only on de novo cholesterol biosynthesis for their growth requirements.[109] Subsequent exposure to a high statin concentration is known to specifically deplete ER cholesterol pool while other cholesterol pools in the cell stay intact.[109] Such an approach revealed that ER cholesterol pool is required for caspase-1 activation and IL-1β and IL-18 secretion (Figure 1).[106] Again, depletion of ER cholesterol only affected the NLRP3 inflammasome without impeding the activation of AIM2 inflammasome.[106] Though the precise cellular localization of NLRP3 is debatable, growing evidence indicates that NLRP3 is partly ER localized.[110-113] In line with cholesterol functions in other membranes, the authors speculated that ER cholesterol provides the necessary fluidity and or conformation for NLRP3 to sense activating stimuli.[106] Regardless, these findings suggest a critical role for cholesterol trafficking and ER cholesterol levels in NLRP3 inflammasome activation.

3 | OXYSTEROL-MEDIATED REGULATION OF NLRP3 INFLAMMASOME

Cholesterol has key roles in maintaining cellular homeostasis by providing fluidity and permeability to biological membranes and by regulating signaling events.[55,83,84,93] As discussed in detail above, cholesterol homeostasis is coordinated by competing transcription factors, SREBP2 and LXRs, which, respectively, coordinate cholesterol biosynthesis and efflux programs in response to the availability of cholesterol and oxysterols.[93,114-117] Recent studies have expanded the functions of oxysterols in immune responses. In particular, numerous functions for 25-hydroxycholesterol (25-HC) have been identified including its roles in the regulation of immune cell migration, differentiation, and modulation of inflammatory signaling.[118-121]

Due to their relatively low-abundance and poor ionization characteristics, oxysterols are often not observed in global lipidomic analysis.[122] Much research describing 25-HC functions, therefore, has relied on cholesterol 25-hydroxylase (Ch25h), a transmembrane ER-localized enzyme, which generates 25-HC by hydroxylation of cholesterol at position 25.[123] However, Ch25h itself is expressed at low-to-undetectable levels at steady state but is robustly produced in response to TLR3 and TLR4 ligation suggesting important functions for both Ch25h and the product 25-HC in innate immunity to pathogens.[120,124] TLR induction of Ch25h requires a TRIF-dependent pathway mediated by type I interferon (IFN) and signal transducer and activator of transcription 1 (STAT1) signaling (Figure 2).[120,124] Though the enzyme is expressed by various cell and tissue types, macrophages and DCs have among the highest expression of Ch25h.[125]

25-HC has been demonstrated to suppress the priming and activation of NLRP3 inflammasome.[126] BMDMs deficient in *Ch25h* display increased transcript levels of *Il1b* in response to TLR4 agonist LPS. Moreover, NLRP3 activation in deficient cells resulted in increased caspase-1 activity and secretion of the mature form of constitutively expressed IL-18. Remarkably, these cells also revealed enhanced caspase-1 levels in response to NLRC4 and AIM2 agonists implying a broader role for Ch25h across multiple inflammasomes.[126] 25-HC mediates its effects *via* two main pathways: by activating LXRs and through its ability to repress SREBP2.[118,127] However, macrophages lacking *Lxrα* and *Lxrβ* did not mimic cells that lacked *Ch25h* in IL-1β production suggesting the involvement of the SREBP2 pathway. Notably, while cholesterol is sensed by SCAP, 25-HC directly binds to INSIG to repress SREBP2 processing. Accordingly, INSIG overexpression in *Ch25h*-deficient cells reduced IL-1β transcription.[126] Moreover, cells lacking the SREBP2 chaperone protein SCAP revealed a modest but significant decrease in caspase-1 levels.[126] In agreement with detrimental roles for unrestricted NLRP3 activity, mice deficient in *Ch25h* exhibited increased susceptibility in mouse models of endotoxin-induced septic shock and experimental autoimmune encephalomyelitis.[126] Moreover, in response to alum-induced peritonitis, *Ch25h*-deficiency elevated neutrophil recruitment compared to that observed in WT mice.[126] Correspondingly, SCAP-SREBP2 complex translocation to the Golgi is required for NLRP3 activation.[128] These studies, therefore, demonstrate the involvement of SREBP2 in 25-HC-mediated restriction of IL-1β processing and advocate novel avenues by which NLRP3 activity may be calibrated.

The ability of 25-HC to restrain inflammasome activation and thus IL-1β production is centered on its ability to relay suppression of cholesterol synthesis. However, feedback inhibition of cholesterol synthesis is only partly mediated by 25-HC.[127,129] For the most part, cholesterol largely regulates its own synthesis through binding to SCAP and preventing SREBP2 translocation from the ER to be processed into the active form.[122] Moreover, other studies have found that CH25H is not an IFN-inducible enzyme in humans, and the deletion of STAT1 does not adversely affect CH25H induction.[124] Finally, mice lacking Ch25h show normal cholesterol metabolism; consequently, the physiological function for 25-HC in cholesterol synthesis and metabolism is unclear at the moment.[118,130,131] Nevertheless, these studies provide a potential link between dysregulation of cholesterol metabolism and inflammasome activation which remains interesting to date.

4 | DYSREGULATED CHOLESTEROL METABOLISM RESULTS IN AIM2 INFLAMMASOME ACTIVATION

Defects in cholesterol regulatory mechanisms can impact additional inflammasomes. Besides modulating the NLRP3 inflammasome, deficiency in *Ch25h* has also been demonstrated to regulate the activation of the DNA sensing AIM2 inflammasome.[131] Due to their inability to suppress SREBP2 processing, cells lacking *Ch25h* accumulate excess cholesterol.[131] Multiple feedback mechanisms exist to resolve surplus cholesterol accumulation, including the

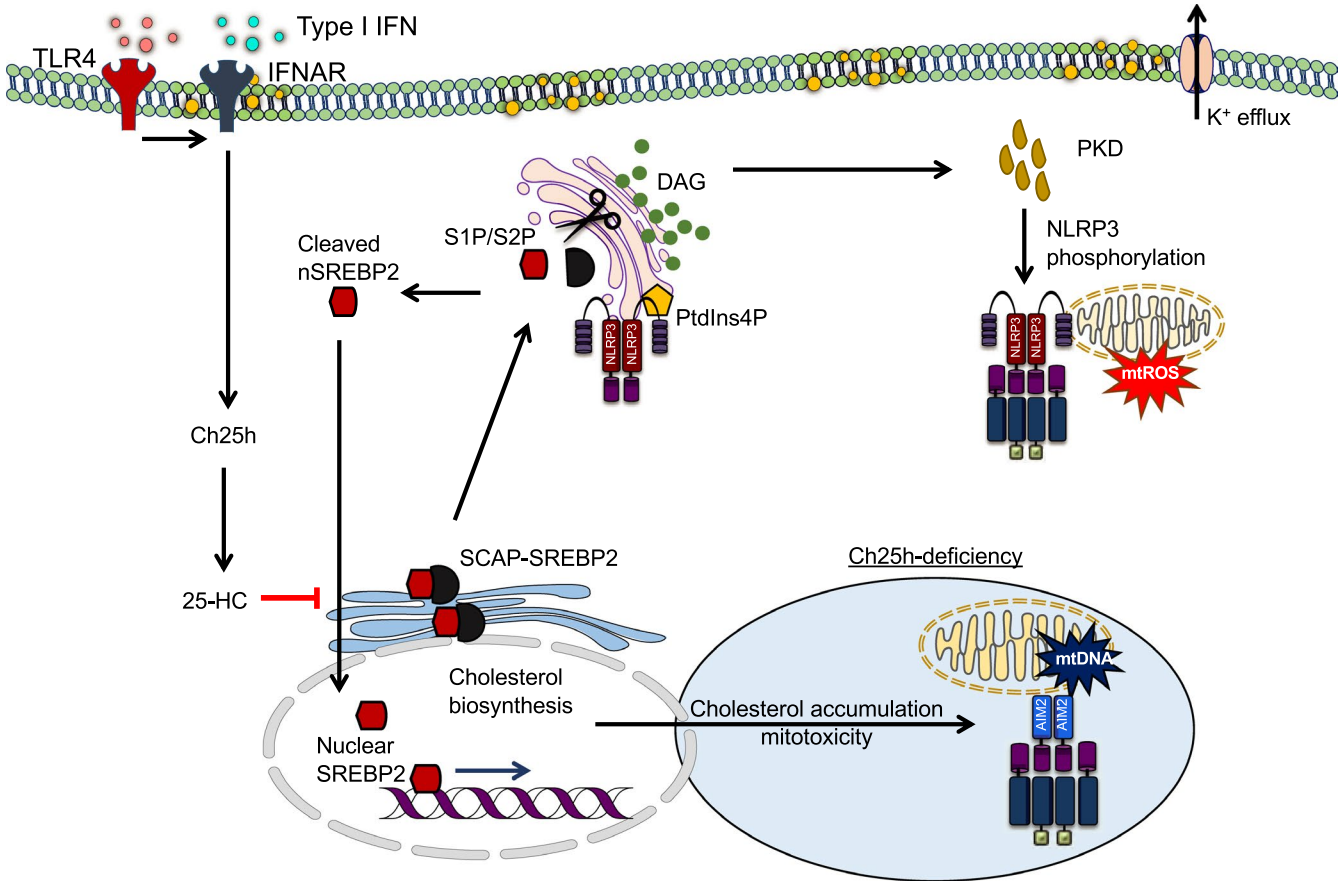


FIGURE 2 Oxysterol-mediated regulation of NLRP3 inflammasome. TLR4 ligation by LPS results in type I IFN production which upregulates the expression of the enzyme Ch25h. Ch25h leads to 25-HC production which inhibits SREBP2-mediated cholesterol biosynthesis. During *Ch25h*-deficiency, this inhibition is lost resulting in SCAP-SREBP2 activation and increased cholesterol biosynthesis. Elevated cholesterol levels increase mitochondrial toxicity resulting in exposure of mtDNA to the cytoplasm thereby activating the AIM2 inflammasome. NLRP3 can also be recruited to the TGN through ionic bonds with PtdIns4P. Accumulation of DAG at the Golgi results in PKD activation which helps phosphorylate NLRP3. NLRP3 phosphorylation is required for activation of the NLRP3 inflammasome at mitochondria-associated membranes. Ch25h, cholesterol 25-hydroxylase; 25-HC, 25-hydroxycholesterol; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; TGN, *trans*-Golgi network; PtdIns4P, phosphatidylinositol-4-phosphate; DAG, diacylglycerol; PKD, protein kinase D

ability to esterify excess free cholesterol by acyl-CoA cholesterol acyltransferase (ACAT) to be stored in lipid droplets.[117,132] Similarly, increased exogenous cholesterol loading is avoided by downregulation of LDL-R expression.[132] However, under conditions when these feedback mechanisms are no longer conducive, cholesterol accumulation can have detrimental effects including damage to the mitochondrial membrane and cellular toxicity.[133,134] In cells lacking *Ch25h*, mitochondrial respiratory capacity is compromised resulting in mitochondrial death and mitochondrial DNA (mtDNA) release into the cytoplasm (Figure 2). Sensing of mtDNA results in AIM2 inflammasome activation[131] (Figure 2). Enforced increase in cholesterol content similarly induces mitochondrial dysfunction resulting in caspase-1 activation and IL-1β secretion in WT cells, but not in cells depleted of either mtDNA or *Aim2*.[131] In another approach, cholesterol loading of WT cells with methyl-β-cyclodextrin revealed a similar increase in inflammasome activation, which was not obstructed by cytochalasin D signifying that this response is independent of any effect of cholesterol crystals on NLRP3 inflammasome. These data imply that cholesterol in its "soluble" form also has the ability to modulate inflammasome activation.[95,106,131] Furthermore, these studies highlight how homeostatic cholesterol metabolism prohibits unwanted activation of the AIM2 inflammasome. These findings are highly relevant during metabolic inflammation where IL-1β functions are detrimental and need to be maintained below a pathologic threshold.[131]

The above studies mostly focused on the production of Ch25h and 25-HC during LPS sensing and revealed how homeostatic cholesterol metabolism by maintaining mitochondrial health prevents AIM2 inflammasome activation. Cholesterol accumulation and associated toxicity can also damage other cellular membranes including nuclear membrane. Consequently, activation of the AIM2 inflammasome because of the decompartmentalization of the nuclear DNA cannot be ruled out. It is worth mentioning that oxidized mtDNA can also activate the NLRP3 inflammasome.[135,136] Though independent mechanisms have been proposed, this is consistent with the role of Ch25h and altered cholesterol metabolism in regulating both

NLRP3 and AIM2 inflammasomes. It will be interesting to investigate whether AIM2 deficiency can blunt some of the pathological features seen in cholesterol-induced inflammation in diverse diseases.

5 | LIPID SECOND MESSENGER, DIACYLGLYCEROL, AND PROTEIN KINASE D PATHWAY PHOSPHORYLATES NLRP3 FOR ACTIVATION

Diacylglycerol (DAG) is a neutral lipid involved in several metabolic pathways and mediates signaling functions as a second messenger.[137] DAG and inositol-1,4,5-triphosphate ($InsP_3$) are produced by the action of phospholipase C on PM lipid, phosphatidylinositol 4,5-bisphosphate ($PtdIns4,5P_2$).[138] DAG is primarily present in the cytoplasm but translocates to the Golgi upon NLRP3 activation.[112] Remarkably, disruption of the Golgi structure diminished NLRP3 activation and IL-1β secretion suggesting a central role for this organelle in inflammasome activation. Activation of the inflammasome specifically required NLRP3 phosphorylation at Ser293 position by DAG effector protein kinase D (PKD) (Figure 2). Mechanistically, phosphorylation by PKD allows NLRP3 to leave mitochondria-associated ER membranes (MAMs) to assemble inflammasome in the cytoplasm. Notably, phosphorylation by PKD occurred after the NLRP3 oligomerized. In agreement, PKD inactivation prohibited the release of oligomerized NLRP3 from MAMs to the cytoplasm and prevented inflammasome activation.[112] Moreover, pharmacological inhibition of PKD in cells from CAPS patients (which show spontaneous NLRP3 activity) reduced NLRP3 activation. Conspicuously, the release of intracellular Ca^{2+} stores from the ER by $InsP_3$, leading to mitochondrial Ca^{2+} overload and mitochondrial damage, also promotes inflammasome activation.[139,140] These studies, therefore, imply important roles for lipid second messengers in NLRP3 activation and highlight complex crosstalk between distinct organelles for effective NLRP3 inflammasome activity.

6 | oxPAPC BINDS TO CASPASE-11 TO TRIGGER IL-1β SECRETION IN THE ABSENCE OF PYROPTOSIS

Cellular inflammatory responses at the site of infection or injury are determined by internal and external cues with cytokines, growth factors, and lipids playing prominent roles.[141,142] The presence of these cues ensures that the cellular response is apt to the initial insult. LPS sensing by immune cells involves the coordination of several receptors.[143-146] The secreted LBP and the GPI-anchored protein CD14 extract LPS from the bacterial cell wall to deliver lipid A to the membrane-associated MD-2 and TLR4 to initiate downstream signaling.[144,146] Besides this conventional signaling by TLR4, LPS is also sensed directly in the cytoplasm by caspase-11 inflammasome (caspase-4/5 in humans) leading to the induction of pyroptosis and IL-1β secretion.[33,34,147] Additionally, CD14-dependent internalization of the LPS, CD14, and TLR4 complex drive an endosomal pathway in the form of IFN-I signaling.[148-150] Recent studies demonstrated that a complex mixture of oxidized lipids released from dying cells, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (oxPAPC), can have profound effects on the quality of the innate immune response.[151-153] oxPAPC by directly binding to CD14 receptor results in CD14 internalization (Figure 3). Intriguingly, the CD14-binding site for oxPAPC is identical to the one used by LPS and involves the same PLCγ and Syk-dependent mechanism for internalization.[148,151] The internalization of CD14 upon oxPAPC binding made cells insensitive to subsequent LPS stimulation, clarifying previously described inhibitory activity of oxPAPC on TLR signaling.[151,154] In contrast, oxPAPC treatment after TLR priming of DCs resulted in oxPAPC endocytosis and translocation into the cell. oxPAPC binding to caspase-11 prompted NLRP3 activation and IL-1β secretion (Figure 3), referred to as "hyperactivation." Notably, this IL-1β secretion occurred in the absence of pyroptosis but specifically required oxPAPC cytoplasmic presence. Accordingly, CD14-deficient cells transfected with oxPAPC still produced similar levels of IL-1β. Moreover, individual oxPAPC components also hyperactivated DCs signifying that the observed response to oxPAPC is a net response to the individual components of this complex lipid mixture. The physiological significance of these results was validated in a mouse model. Mice injected with LPS followed by oxPAPC exhibited increased IL-1β levels suggestive of a hyperactivation phenotype but they neither presented pyroptosis nor succumbed to death. By contrast, mice injected with two subsequent doses of LPS succumbed due to sepsis involving both IL-1β secretion and pyroptotic phenotype. This research thus proposes a key role for lipids in modulating IL-1β secretion and sheds light on the intricate mechanisms by which immune responses to infectious and sterile stimuli are calibrated.

7 | FATTY ACIDS MODULATE THE INFLAMMASOME ACTIVATION DURING TYPE 2 DIABETES

Elevated plasma fatty acid levels, mainly a result of increased high-fat diet consumption, contribute to the development of several human diseases including type 2 diabetes (T2D).[155,156] Despite evidence that chronic low-grade inflammation underlies insulin resistance, a hallmark of T2D, the mechanistic details and the involvement of inflammatory mediators remain unresolved. Earlier studies implicated elevated glucose levels and islet β-cell dysfunction in IL-1β production during T2D.[157,158] These studies, however, could not fully explain a role for the inflammasome and IL-1β secretion in T2D pathogenesis. Studies from the Ting laboratory demonstrated that the fatty acid palmitate (C16:0; a 16-carbon saturated fatty acid), one of the abundant fatty acids in human plasma,[156] can activate the NLRP3 inflammasome[159] (Figure 3). Exposure to BSA-conjugated palmitate following LPS priming of macrophages and DCs resulted in enhanced secretion of IL-1β and IL-18[159] (Figure 3). In their model, IL-1β release by hematopoietic cells could decrease insulin signaling

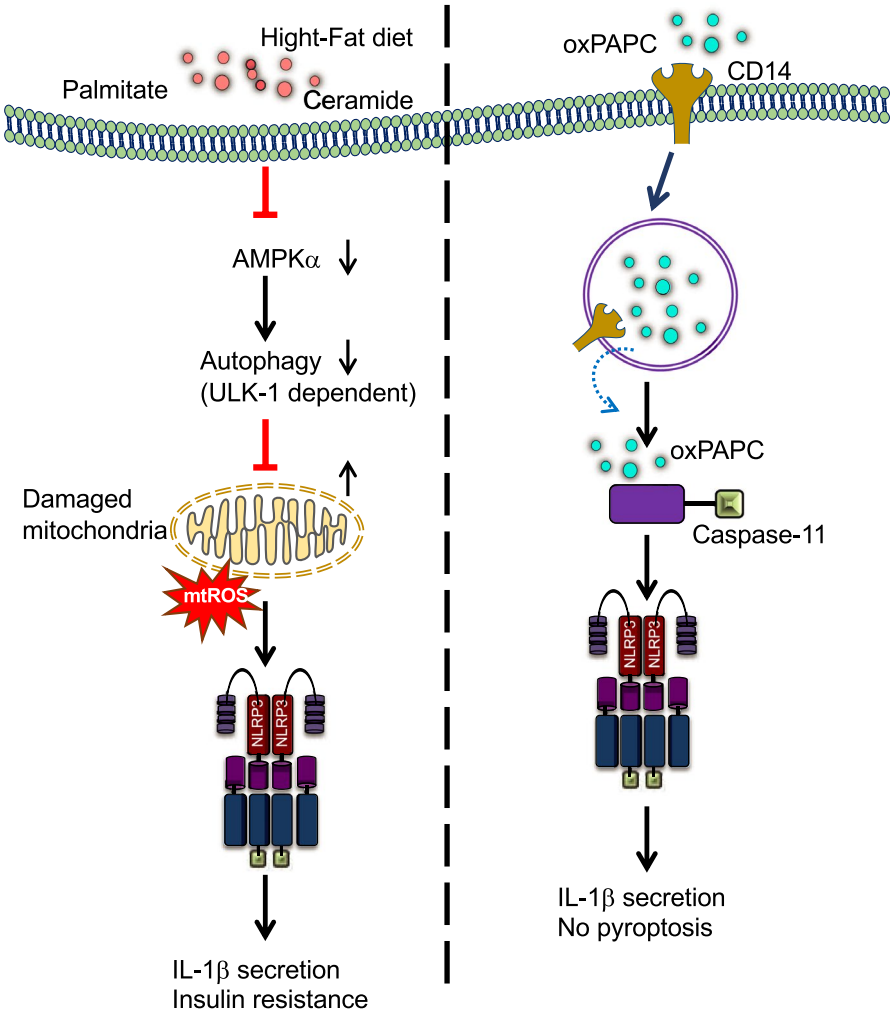


FIGURE 3 Fatty acids and oxidized phospholipids activate the NLRP3 inflammasome. *Left panel*, Fatty acids such as palmitate inhibit AMPK phosphorylation which is required for autophagic degradation of mitochondria in an ULK1-dependent manner. In the absence of mitophagy, damaged and dysfunctional mitochondria persist leading to elevated levels of mtROS which activates the NLRP3 inflammasome resulting in IL-1β secretion and increased insulin resistance during type 2 diabetes. Small arrows denote the upregulation or downregulation of an event upon stimulation with palmitate. *Right panel*, Oxidized phospholipids such as oxPAPC are endocytosed by a CD14-dependent mechanism. Within the cell, oxPAPC translocates to the cytoplasm and directly binds caspase-11 leading to NLRP3 inflammasome activation and IL-1β secretion. Of note, this phenotype, known as hyperactivation, proceeds independently of pyroptosis. AMPK, adenosine monophosphate (AMP)-activated protein kinase; ULK1, unc-51-like kinase 1; mtROS, mitochondrial reactive oxygen species; oxPAPC, oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine

in vitro. Consequently, treatment of a mouse liver cell line with IL-1β decreased the phosphorylation of serine-threonine kinase AKT reflecting dampened insulin signaling. TNF-α has previously been implicated in insulin resistance.[160] Indeed, inhibition of AKT phosphorylation could also be achieved by TNF-α implying that both the cytokines have the ability to impart insulin resistance. In agreement, both IL-1β and TNF-α induced phosphorylation of insulin receptor substrate-1 (IRS1), an upstream event in insulin resistance. These results also recapitulated in vivo as IL-1β administration promoted insulin resistance more sharply in WT mice than *Tnfα*$^{-/-}$ mice suggesting the existence of both TNF-dependent and TNF-independent pathways of insulin resistance.[159]

Mechanistically, palmitate inhibits the phosphorylation and activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK) which is known to directly regulate autophagy by phosphorylation-dependent activation of unc-51-like kinase 1 (ULK1, the mammalian homolog of yeast ATG1)[161,162] (Figure 3). In the presence of aberrant autophagy, cells exposed to palmitate display increased levels of mtROS, a recognized stimulus for NLRP3 inflammasome activation (Figure 3). Consequently, exposure to AMPK activator AICAR or transfection with constitutively active AMPK-α1 restored autophagy by inducing ULK1 phosphorylation at Ser467 and Ser555 position, resulting in decreased mtROS levels and diminished IL-1β secretion.[159] Besides playing key roles in several metabolic pathways and in the maintenance of cellular energy homeostasis, these studies implicate AMPK in NLRP3 regulation thereby highlighting tight links between metabolism and inflammasomes.

One recent report has suggested that saturated fatty acids such as palmitic acid and stearic acid (C18:0) undergo crystallization and thus activate the NLRP3 inflammasome through lysosomal destabilization.[163] Furthermore, palmitic and stearic acid-induced NLRP3 activation could be inhibited in the presence of oleic acid (C18:1), a mono-unsaturated fatty acid. Notably, this latter report demonstrated that though saturated fatty acids induced ROS production, it was insensitive to and failed to inhibit IL-1β release in response to mtROS inhibitor, mitoTEMPO. The differences in the two reports could be because of the source and concentration of fatty acids used, or differences in cell types employed in the two studies.[159,163]

Increased levels of saturated fatty acids promote the synthesis of lipid species such as ceramides and DAG.[164] Ceramide production is associated with an inflammatory response during obesity-induced diabetes.[165-167] In agreement, stimulation of LPS-primed BMDMs with ceramide results in caspase-1 cleavage and IL-1β secretion[168] (Figure 3). Elevated NLRP3 activity in adipose tissue macrophages, in turn, triggers T cell activation and, thereby, impairs insulin sensitivity. Accordingly, *Nlrp3*$^{-/-}$ mice reveal increased PI3K-AKT signaling

compared to obese WT mice in response to insulin administration. Moreover, this accompanied reduced phosphorylation of IRS1 in liver and fat of *Nlrp3*$^{-/-}$ obese mice.[168] However, because of its sensitivity to several DAMPs, NLRP3 inflammasome can also be activated by other pathways during obesity. Notably, obesity leads to hypoxia and cell death in the adipose tissue,[169-172] and several endogenous molecules released as a consequence of cell death have the ability to activate the NLRP3 inflammasome. This signifies how complex an in vivo response could be and that the balance in locally available metabolites and DAMPs shape the net inflammasome activity in tissues.

8 | NLRP1-IL-18 AXIS PREVENTS OBESITY AND METABOLIC SYNDROME

Excess body weight, often a result of dependence on a high-fat western diet, is a major risk factor for diabetes. Obesity-associated type 2 diabetes accounts for more than 90% of all diabetes cases in adults worldwide.[173] Diabetes and insulin resistance are also strong predictors of cardiovascular disease and are associated with altered metabolism characterized by dyslipidemia and hyperglycemia.[173] The concept that inflammation leads to metabolic diseases has been around for quite some time, but definitive evidence came from studies revealing elevated TNF-α mRNA within adipose tissue of rodent models of obesity, and that TNF-α neutralization improved insulin sensitivity.[174] Besides the roles of IL-1β in diabetes (discussed earlier), IL-18 has also been implicated. Obese individuals exhibit elevated serum IL-18 levels that correspond with insulin resistance.[175] Paradoxically, IL-18 administration prevents weight gain in mice, while *Il18* deficiency raised adiposity and insulin resistance.[176,177] Other studies have rationalized this by proposing that IL-18 elicits AMPK signaling and lipid oxidation in the skeletal muscle thereby balancing lipid accumulation on a high-fat diet.[178] However, chronic IL-18 production during obesity leads to the tolerization of the pathway by downregulating IL-18 receptor expression, which could explain consistently higher serum IL-18 levels in obese individuals.[179,180] Increase in obesity in *Il18*$^{-/-}$ mice is phenocopied by those lacking *Nlrp1* suggesting the involvement of this inflammasome in IL-18 dependent prevention of adiposity and metabolic syndrome. In agreement, mice with NLRP1 activating mutation exhibited decreased adiposity and resistance to diet-induced metabolic dysfunction.[180] Therefore, NLRP1-IL-18 axis protects against metabolic disorder, and under certain settings, NLRP1 may act as a sensor of cellular homeostasis.

9 | OTHER LIPID-MEDIATED PATHWAYS IN INFLAMMASOME ACTIVATION

9.1 | ω-3 fatty acids negatively regulate inflammasomes

Omega-3 (ω-3) polyunsaturated fatty acids, which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have varied roles in human health and disease.[181] Deficiency in ω-3 fatty acids is associated with chronic diseases. One study demonstrated that pretreatment of LPS-primed BMDMs with DHA and EPA inhibited inflammasome activation and IL-1β secretion.[182] Remarkably, the ω-3 fatty acids inhibited activation of both the NLRP3 and NLRP1b inflammasomes.[182] However, activation of the NLRC4 and AIM2 inflammasomes was not affected. Anti-inflammatory effects of ω-3 fatty acids may be mediated through certain enzymatic oxygenated products such as resolvin D1 and protectin D1.[183-185] However, these enzymatic products did not affect IL-1β secretion in response to NLRP3 activation. Mechanistically, the ω-3 fatty acids inhibit NLRP3 and NLRP1b inflammasomes by a pathway involving G protein-coupled receptors, GPR120 and GPR40, acting upstream of β-arrestin 2. Sequestration of NLRP3 by β-arrestin 2 in THP-1 cells inhibits the inflammasome activity. Finally, in a mouse model of T2D, supplementation with ω-3 fatty acids improve insulin sensitivity as observed by AKT(Ser473) phosphorylation. These studies, therefore, demonstrate the ability of ω-3 fatty acids to inhibit inflammasomes and could be exploited for therapeutic benefits in T2D and other chronic diseases.

9.2 | Phosphatidylinositol 4-phosphate recruits NLRP3 to the Golgi during activation

Phosphoinositide (PI) lipids exist as mono, bis-, or tris-phosphorylated derivatives of the glycerophospholipid phosphatidylinositol (PtdIns). The discrete subcellular localization of PtdIns phosphatases and PtdIns kinases determines the accumulation of PtdIns in defined subcellular sites.[186,187] Phosphatidylinositol 4-phosphate (PtdIns4P) is present in multiple subcellular sites including the PM and the Golgi,[188,189] and has been recently demonstrated to contribute to NLRP3 inflammasome activation.[190] By developing an in vitro assay to examine NLRP3 activation, the authors demonstrated that GFP-tagged NLRP3 formed multiple puncta at the *trans*-Golgi network (TGN) before oligomerizing with ASC protein. They reasoned that the dispersion of the *trans*-Golgi network (dTGN) is the earliest event that is required for the NLRP3 activation in response to diverse NLRP3 stimuli.[190] The recruitment of NLRP3 to dTGN specifically required four consecutive lysine residues between the PYD and the NACHT domain of NLRP3, and it is this polybasic sequence of NLRP3 that forms ionic bonds with PtdIns4P pool present in the dTGN (Figure 2). In contrast to many previous studies, NLRP3 was not observed to co-localize with mitochondria and instead dTGN served as the scaffold for NLRP3 activation. These studies advance our understanding of the activation mechanisms of NLRP3 inflammasome which await further confirmation.

9.3 | NLRP3 and IL-1β secretion affect adipogenesis

Activation of the NLRP3 inflammasome is associated with adipocyte differentiation and adipogenesis. Exposure of adipose tissue

mesenchymal stem cells (MSCs), which have the ability to differentiate to multiple cell types including adipocytes and osteocytes, to NLRP3 activating stimuli resulted in increased adipogenesis but decreased osteogenesis.[191] Consequently, caspase-1 inhibition suppressed adipogenic but improved osteogenic differentiation.[191] Activation of caspase-1 is associated with induction of pro-adipogenic factor PPAR-γ while it hinders the expression of pro-osteogenic bone morphogenic protein 2 and runt-related transcription factor 2. In agreement, caspase-1 has been proposed as a potential biomarker and target in osteoporosis,[191] a skeletal disease characterized by osteopenia and adipose accumulation in bone tissue. Other studies have demonstrated that elevated caspase-1 during adipocyte differentiation imparts insulin-resistant phenotype to these cells.[192] Such effects were observed to be largely conveyed by IL-1β. Accordingly, *Casp1*$^{-/-}$ mice, or obese WT mice treated with the caspase-1 inhibitor, exhibit increased sensitivity to insulin.[192] Moreover, ultrastructure studies revealed active caspase-1 and pyroptosis in hypertrophic obese adipocytes.[193] Finally, inhibition of NLRP3 in human visceral adipocytes attenuated adipose tissue fibrosis.[194] These studies propose that caspase-1 activation and IL-1β production affect multiple facets of adipocytes.

9.4 | AIM2 inflammasome mediates complex functions in metabolic disorders

In contrast to the NLRP3 function, deficiency in AIM2 increases inflammation and adipogenesis leading to spontaneous obesity and insulin resistance.[195] Correspondingly, *Aim2*$^{-/-}$ mice were more obese than their WT counterparts and exhibited reduced energy expenditure and higher glucose intolerance.[195] Moreover, *Aim2*$^{-/-}$ mice exhibited upregulation of IFN-inducible IFI202b and elevated inflammatory signaling in adipose tissue. Of note, *Ifi202b* knockdown blocked adipogenesis in stromal vascular fractions.[195] These studies, therefore, imply important functions for AIM2 and IFI202b in inflammation and insulin resistance. However, other studies have revealed that AIM2 activation by circulating cell-free mitochondrial DNA might contribute to chronic inflammation in type 2 diabetes.[196] AIM2 is also a regulator of hepatic fat levels. Deficiency in *Aim2* resulted in liver enlargement with increased lipid deposition.[197] This corresponded with higher expression levels of lipogenic genes such as *Acc1*, *Fasn*, and *Srebp-1c* in the absence of *Aim2*.[197] These studies thus proposed novel therapeutic avenues for the treatment of non-alcoholic fatty liver disease. Together, these studies advocate important functions for AIM2 in metabolic disorders.

9.5 | Lipid biomarkers and NLRP3 inflammasome in gouty nephropathy

In a study to identify potential biomarkers of gouty nephropathy, plasma metabolites were identified by ultra-performance liquid chromatography.[198] The patients' peripheral blood mononuclear cells (PBMCs) exhibited elevated expression of NLRP3 and further displayed elevated plasma IL-1β and IL-18 levels compared to the control group. The study identified several potential plasma metabolic biomarkers, the majority of which were involved in lipid metabolism, particularly those that regulated the activity of phospholipase A2 and β-oxidation.[198] The authors concluded that lipid metabolism and NLRP3 inflammasome play pivotal roles and may be involved in the progression of gouty nephropathy.

10 | FUTURE PERSPECTIVE AND CONCLUSIONS

The integral roles of lipid metabolism in inflammasome activation have recently gained much attention. New studies have described the key functions of cholesterol synthesis and transport, dysregulation in oxysterol production, fatty acid signaling, and other critical mechanisms by which lipids regulate inflammasome activity. In particular, cholesterol metabolism has been in focus due to the unraveling of numerous pathways by which it regulates caspase-1 activation and IL-1β production. Cholesterol trafficking, the role of lysosomal cholesterol transporter NPC1, the involvement of SREBP2, and oxysterol-mediated regulation of mitochondrial health have all added an exciting direction to the inflammasome research.

While the localization of individual components and subsequent inflammasome assembly remains debatable, key involvement for ER and mitochondria-associated ER membranes have been recognized. In this context, ER is also the site for cholesterol biosynthesis and is entrusted with the task of sensing and swiftly responding to maintain cellular cholesterol levels within an exceedingly tight range. Cholesterol is also established to regulate diverse inflammatory responses. Therefore, in terms of energy efficiency, it is reasonable that the liability to regulate inflammasome activation, a highly inflammatory state which advances to cell death, is tied to the same mechanisms that uphold cellular cholesterol levels. Indeed, ER cholesterol pool has been shown to regulate the activation of the NLRP3 inflammasome. However, further elucidation of these pathways is required before therapeutic avenues can be considered as this could pose detrimental inflammation if dysregulated.

NLRP3 can respond to the presence and change in the composition of different metabolites and other cues in the local tissue environment. These studies have strengthened the idea that NLRP3 is a general sensor of cellular homeostasis, Indeed, perturbations in diverse metabolic pathways are all known to activate the NLRP3 inflammasome.[199-201] While cellular metabolic rewiring resulting in the secretion of cytokine mediators is beneficial in the interim, inability to resolve the inciting stimuli or revert to the steady state may, however, lead to chronic inflammation. Of note, it is recognized that inflammasome-induced pyroptosis and IL-1β secretion as a result of altered lipid metabolism contribute to several pathologies including atherosclerosis and diabetes. Notably, IL-1β and IL-18 play contrasting roles in distinct diseases including obesity-associated diabetes. Therefore, the development of therapeutics that target

specific inflammasome functions is required so that the beneficial outcomes of inflammasomes can be retained. Furthermore, unraveling the roles of lipids in inflammasome activation and IL-1β secretion will aid in the development of therapeutics to limit inflammation-related pathologies in several diseases including where perturbed lipid metabolism is observed.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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