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#### REVIEW

# Inflammasome activation: from molecular mechanisms to autoinflammation

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#### Abstract

Inflammasomes are assembled by innate immune sensors that cells employ to detect a range of danger signals and respond with proinflammatory signalling. Inflammasomes activate inflammatory caspases, which trigger a cascade of molecular events with the potential to compromise cellular integrity and release the IL-1 $\beta$ IL-18 pro-inflammatory cytokines. Several molecular and mechanisms, working in concert, ensure that inflammasome activation is tightly regulated; these include NLRP3 posttranslational modifications, ubiguitination and phosphorylation, as well as single-domain proteins that competitively bind to key inflammasome components, such as the CARD-only proteins (COPs) and PYD-only proteins (POPs). These diverse regulatory systems ensure that a suitable level of inflammation is initiated to counteract any cellular insult, while simultaneously preserving tissue architecture. When inflammasomes are aberrantly activated can drive excessive production of pro-inflammatory cytokines and cell death, leading to tissue damage. In several autoinflammatory conditions, inflammasomes are aberrantly activated with subsequent development of clinical features that reflect the degree of underlying tissue and organ damage. Several of the resulting disease complications may be successfully controlled by anti-inflammatory drugs and/or specific cytokine inhibitors, in addition to more recently developed small-molecule inhibitors. In this review, we will explore the molecular processes underlying the activation of several inflammasomes and highlight their role during health and disease. We also describe the detrimental effects of these inflammasome complexes, in some pathological conditions, and review current therapeutic approaches as well as future prospective treatments.

Keywords: autoinflammatory disorders, inflammasome, inflammation, NLRC4, NLRP3, Pyrin

# INTRODUCTION

Innate immune cells constitute the first line of defence against invading pathogens, conferring host protection through several multidimensional non-specific molecular strategies. Activation of these pathways normally occurs via detection of danger-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), which is an integral component of Gram-negative bacteria outer membranes. LPS is recognised by several different pattern-recognition receptors (PRRs),<sup>1</sup> which induce a specific response to counteract each individual situation and the particular invading organism encountered. For instance, phagocytes can detect opsonised molecules, via their opsonic receptors, and these cells will eventually phagocytose the targeted molecule. In situations where the insult overcomes the initial innate defence response, a more complex, robust and damaging mechanism may be induced by activating the inflammasome. The inflammasome was first described, in 2002, by Martinon et al. as a 'high molecular weight complex containing NALP1, Pycard, and proinflammatory caspases'<sup>2</sup>; as then several more studies have elucidated several distinct inflammasomes that can be assembled through activation of other cytosolic PRRs.<sup>3,4</sup> In general, inflammasomes are activated by two consecutive signals; the first signal arises from Tolllike receptors (TLRs) that are activated by a PAMP such as LPS, which triggers the production of inactive forms of two pro-inflammatory cytokines, interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-18 (IL-18), known as pro-IL-1 $\beta$  and pro-IL-18.<sup>2</sup> Cytokines such as TNF or even IL-1 itself can also provide this first signal.<sup>5</sup> The second signal can arise from multiple pathways (e.g. K<sup>+</sup> efflux via P2X7 receptor activation, endoplasmic reticulum stress) and results in the activation of proinflammatory caspases, which in turn proteolytically activateIL-1ß and IL-18 for secretion.<sup>6</sup> Although caspase-1 activation is essential to the inflammasomemediated cleavage of both pro-IL-1 $\beta$  and pro-IL-18, a number of different proteases, apart from caspase-1, may also cleave the pro-forms of IL-1 $\beta$ and IL-18 at different sites, thereby giving rise to peptide/protein fragments with noninflammasome-related biological activity.<sup>6,7</sup> Nevertheless, not all inflammasomes need the priming signal to become fully activated and they can induce the secretion of inflammatory cytokines through other mechanism.

Several inflammasomes with a range of different PRRs, functions and regulators are now described.<sup>3,4</sup> Generally speaking, 'canonical' inflammasomes can be classified according to the three different categories of PRRs that nucleate these protein complexes; these are NOD-like receptors (NLRs), pyrin and AIM2-like receptors (ALRs) (Figure 1). Inflammasome sensor activation is usually followed by recruitment of the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC).<sup>8</sup> Finally, the effector molecule, caspase-1, is recruited to the complex bv ASC to form the large multimeric inflammasome complex.<sup>9</sup> Inflammasomes usually employ caspase-1 as their effector protein; however, 'non-canonical inflammasomes' are also described, which activate other caspases, such as caspases-11/4/5.<sup>10</sup> These specialised 'non-canonical' inflammasomes can detect intracellular LPS inducing inflammatory responses and pyroptosis.<sup>11</sup>

While detection of PAMPs and DAMPs by specific inflammasome sensor proteins is usually followed by a pro-inflammatory response, two other single-domain protein molecular families, referred to as CARD-only proteins (COPs) and pyrin-only proteins (POPs), have emerged as negative regulators of inflammasomes<sup>12</sup> (Figure 1). These short structures are important inflammasome regulators and play a key role in fine-tuning the inflammatory response.

Inflammasomes are vital innate immune defence complexes, responsible for monitoring pathogenic invasions and counteracting molecular insults. Perturbations of their signalling mechanisms may lead to immunological conditions<sup>13,14</sup> and genetic disorders.<sup>13,15</sup> Autoinflammatory diseases, such as tumor necrosis factor receptor-associated periodic syndrome (TRAPS) and familial mediterranean fever (FMF), have all been directly linked with excessive production of IL-1 $\beta$  and IL-18,<sup>14</sup> because of heightened inflammasome responses. Therefore, a selection of these conditions will also be



**Figure 1.** Domain structure of inflammasomes and regulators. Different types of inflammasomes are shown; Nucleotide-binding and oligomerisation domain NOD-like receptors (NLRs): NLRP1, NLRP3, NLRP6, NLRC4 and NLRC5 all contain a central NACHT domain and a and C-terminal leucine-rich repeats (LRRs) domain; NLRP1, NLRP3, NLRP6 encompass an N-terminal Pyrin domain (PYD); NLRP1, NLRC4 and NLRC5 contain a caspase recruitment domain (CARD); only the human NLRP1 contains a function-to-find domain (FIIND). Pyrin inflammasome consists of a PYD, a B-box (BB), a coiled-coil (CC), and a B30.2 domain. The AIM2-Like Receptors (ALR) AIM2 and IF116 are members of PYHIN family, containing an N-terminal PYD and C-terminal HIN-200 domain. PYD-only proteins (POPs) and CARD-only proteins (COPs) are composed of PYD or CARD domains, accordingly, with different amino acid (AA) composition. The adaptor protein ASC links inflammasome sensors and caspase-1 via the PYD and CARD domains.

discussed, from the molecular level to the clinical picture, including current and potential future treatments.

# INFLAMMASOMES SIGNALLING PATHWAYS

While inflammasome activation may vary in character, depending on the inflammasome in question, the activation process essentially involves four key steps overall – priming, sensing, oligomerisation and activation/release of inflammatory cytokines<sup>16</sup> (Figure 2). In the priming step, PAMPs and/or DAMPs induce transcriptional upregulation of specific components of the inflammasome, such as caspases, pro-IL-1 $\beta$  and pro-IL-18.<sup>17</sup> This first priming step is a 'get-ready' signal for the cell to be alert in case a specific inflammasome requires activation. If the cell is subject to a sensing signal, then inflammasomes sense intracellular insults and this is followed by a third step involving inflammasome assembly. Finally, the whole process culminates in release of pro-inflammatory cytokines through membrane pores formed by the pore-forming protein, gasdermin D (GSDMD), while simultaneously inducing pyroptosis. While pyroptosis is a consequence of inflammasome activation, other types of cell death, such as apoptosis, necroptosis and PANoptosis, have also been associated with

inflammasome activation<sup>16-18</sup> (Figure 2). As an example, the NLRP3 inflammasome activation is shown, nevertheless other inflammasomes may signal exactly like the NLRP3 and these will be described in their sections. For example, LPS can function as one of the first priming steps in certain inflammasomes, by inducing the transcription of inflammasome components, such as NLRP3, caspase-1, pro-IL-1 $\beta$  and pro-IL-18. On subsequent detection of specific DAMPs or PAMPs, such as ATP, urate crystals, bacterial-, viral- or fungal- derived components, oligomerisation of the NLRP3 inflammasome occurs with binding of NIMArelated kinase 7 (NEK7) and subsequent recruiting of ASC and caspase-1 to facilitate dimerisation, cleavage and activation of caspase-1.<sup>16</sup> Active caspase-1 induces the cleavage of inflammasomespecific cytokines, such as IL-1 $\beta$  and IL-18, and directs their secretion via pores in the plasma membrane generated by GSDMD, inducing pyroptosis<sup>19,20</sup> (Figure 2).

While the activation mechanism of the inflammasomes aforementioned is well accepted within the majority of them, several other mechanisms exist that can induce inflammasome assembly and caspase activation. For instance, NLRP1 and CARD8 can undergo post-translational autoproteolysis inducing inflammasome activation and release of IL-1 $\beta$  and IL-18 ASC-independently.<sup>21,22</sup> Similarly, NLRC4 can directly



**Figure 2.** NLRP3 inflammasome activation. Danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs) are able to induce the priming step inducing the transcriptional upregulation of inflammasome components. DAMPs or PAMPs are subsequently sensed inducing oligomerisation of the inflammasome sensor, NLRP3. Caspase-1, NEK7, ASC and the NLRP3 all together form the NLRP3 inflammasome. The NLRP11bound to ASC is required for NLRP3 oligomerisation and ASC polymerisation. The inflammasome is activated and active caspase-1 cleaves pro-IL-1β and pro-IL-18 to their mature forms IL-1β and IL-18 which get secreted through gasdermin D pores (GSDMD); alternatively, IL-1β and IL-18 can be secreted via different mechanism avoiding GSDMD.

interact with caspase-1 via its CARD domain, causing protease activation and release of proinflammatory cytokines.<sup>23</sup> Furthermore, these ASC-independent events induce caspase-1 activation, and these processes are enhanced by a priming step which induces ASC and other caspase substrates.<sup>23,24</sup>

#### NLRP1

The first inflammasome to be described contained NOD-, LRR- and pyrin domain-containing protein 1 (NLRP1).<sup>2</sup> This inflammasome contains a unique function-to-find domain (FIIND), located after its LRR domain (Figure 1). FIIND is a highly conserved protein region present in the NLRP1 and CARD8, and its function is still to be fully elucidated<sup>2,25</sup>; however, a study has shown that FIIND is a type of ZU5- and UPA-like domain contained in autoproteolytic proteins, which undergo autocleavage.<sup>26</sup> The FIIND domain of NLRP1 interacts with dipeptidyl peptidases 8 and 9 (DPP8 and DPP9), suppressing NLRP1 activation.<sup>27</sup> DPP8 and DPP9 are cytosolic prolyl peptidases, with the ability to cleave proline bonds from the Nterminus of substrates, important in the regulation of immune cell functions. The NLRP1 and DDP9 proteins form a complex containing the inhibited NLRP1 molecule and an active UPA-CARD NLRP1 fragment<sup>28</sup>; the ZU5 domain is essential for both, formation of the complex and autoinhibition of NLRP1.<sup>28</sup> NLRP1 haplotype single-nucleotide polymorphisms (SNPs) (M1184V) were associated with reduction of inflammasome activation, via increased binding of DPP9 to the mutated FIIND domain, and this was associated with increased asthma severity.<sup>29</sup> Another unique characteristic of the NLRP1 inflammasome is the location of its CARD domain in its C-terminal region, where it activates caspase-1. Although the NLRP1 inflammasome can be found in most cell types and tissues, it appears to have a vital role in barriers. particularly epithelial the in integumentary and respiratory systems.<sup>30-32</sup>

The recently described CARD8 inflammasome, containing a short N-terminal region, a FIIND, and a CARD domain, can also trigger pyroptosis in CD4<sup>+</sup> and CD8<sup>+</sup> T cells regulated by DPP9.<sup>33</sup> Furthermore, CARD8 was shown to detect HIV-1 protease activity and induce CARD8-depedent pyroptosis in infected T cells and macrophages.<sup>34</sup> The NLRP1 and CARD8 inflammasomes are repressed by DPP8/9 via different mechanisms.<sup>35</sup> A recent study shows that a small molecule named CQ31 can specifically activate CARD8 via inhibition of M24B aminopeptidases prolidase (PEPD) and Xaa-Pro aminopeptidase 1 (XPNPEP1).<sup>35</sup> This inhibition leads to accumulation of prolinecontaining peptides that repress DPP8/9 inducing CARD8 activation.

NLRP1 detects multiple viral protease activities<sup>36,37</sup> in addition to detecting double-stranded (ds) RNA, via its LRR domain, and effectively sensing virus-associated nucleic acids.<sup>38</sup>

Both human and mouse NLRP1 are cleaved in a similar manner, leading to degradation of the N-terminal fragment, with liberation of the C-terminal fragment containing the CARD domain, a potent activator of caspase-1.<sup>36,39</sup> In this way, NLRP1 is seen to be a potent inducer of IL-18 and IL-1 $\beta$ , and fundamental to host protection against certain viruses, mainly in epithelial barriers.

Germline mutations in NLRP1 have been reported to be responsible for two overlying monogenic skin disorders, multiple self-healing palmoplantar carcinoma (MSPC) and familial keratosis lichenoides chronica (FKLC).<sup>30</sup> In that particular study, the reported NLRP1 mutations were gain-of-function mutations, predisposing epithelial cells to NLRP1 inflammasome activation, and augmenting the levels of IL-1B, which exerted paracrine signalling effects leading to multiple epithelium-associated clinical symptoms.<sup>30</sup> MSPC and FKLC both demonstrate classical features of autoinflammation, with spontaneous activation of inflammatory pathways which, in theory, could be resolved by IL-1ß inhibition. Moreover, a total of 23 NLRP1 SNPs have been associated with the vitiligo phenotype with associated autoimmune and/or autoinflammatory features.<sup>31</sup> Intriguingly, only gain-of-function mutations have been reported, so far, in relation to the NLRP1 inflammasome, with another study presenting two cases of juvenileonset recurrent respiratory papillomatosis, with high levels of IL-18, but not of IL-18, in patients' serum.<sup>32</sup> Discrepancies in IL-18 and IL-1β cytokine profiles are observed in other autoinflammatory disorders, with potential divergent roles regarding the site of inflammation.<sup>40,41</sup> These IL-18 and IL-1 $\beta$ disparities can be clinically relevant for the selective treatment of certain inflammatory symptoms observed in some autoinflammatory conditions via small-molecule drugs. Although NLRP1 was the first inflammasome to be described, several questions remain regarding its function and role in other immune-related disorders.

# NLRP3

The NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome plays a critical role in host defence against microbial infections and a multitude of other harmful stimuli in response to endogenous stress. NLRP3 is tightly regulated by post-transcriptional and post-translational modifications and a variety of endogenous modulators.<sup>42,43</sup> Under aberrant

conditions, the NLRP3 inflammasome is associated with many autoinflammatory and metabolic disorders, including cryopyrin-associated periodic syndromes (CAPS), myelodysplastic syndromes (MDS), obesity, type-2 diabetes and Alzheimer's disease.<sup>44</sup>

It is generally thought that effective NLRP3 inflammasome function requires both 'priming' and activation, as described above (Figure 2); however, a recent study found that a priming step may be dispensable in human monocytes, but essential in monocyte-derived macrophages. 45,46 These findings are worth considering while developing specific inflammasome inhibitors, for the purpose of targeting specific steps in inflammasome activation. NLRP3 inflammasome activation involves TLR signalling to activate NF-KB and induce pro-IL-1 $\beta$  and NLRP3 expression, and subsequent activation of the NLRP3 inflammasome by plasma membrane disruption (for bacterial toxins) or by internalisation of particulate activators, such as alum or monosodium urate crystals by phagocytosis.<sup>43</sup> NLRP3 undergoes conformational changes upon activation, mediated by RACK1 and binding to NEK7.47 This enables oligomerisation, providing a platform for ASC recruitment, via PYD-PYD interactions.<sup>47</sup> ASC subsequently polymerises to form large cytoplasmic filaments called ASC specks, and recruits caspase-1 (through its CARD domain), which undergoes dimerisation, self-cleavage at the interdomain linker, and subsequent increase in caspase-1 catalytic activity.<sup>48</sup> This domino effect results in cleavage, maturation and secretion of IL-1 $\beta$ , IL-18, as well as liberation of GSDMD's N-terminal domain, to initiate pyroptotic cell death (Figure 2).

Despite extensive NLRP3 inflammasome characterisation, the precise mechanisms of activation and regulation remain unknown: nevertheless, potential therapeutic targets are constantly emerging.<sup>49,50</sup> The cGAS-STING pathway was recently shown to recruit NLRP3 at the endoplasmic reticulum upon HSV-1 infection, with subsequent NF- $\kappa$ B and NLRP3 activation.<sup>51</sup> Specific blockade of the NLRP3 inflammasome, but not the AIM2 or NLRC4 inflammasomes, by itaconate and its derivative 4-OI, leads to modification of cysteine 548 in the NLRP3 molecule, thereby interfering with NLRP3-NEK7 interactions.<sup>52</sup> Itaconate is a potent anti-inflammatory and anti-microbial metabolite, produced by immune-responsive gene 1 (IRG1) enzyme,<sup>52,53</sup> and itaconate-depleted Ira1<sup>-/-</sup> macrophages show increased NLRP3 activation.<sup>52</sup>

There is increasing evidence of the NLRP3 inflammasome's role in sensing bacterial toxins and virulence factors and activating host Rho GTPases. p21-activated kinases 1 and 2 (Pak1/2) and the Pak1-mediated phosphorylation of Thr-659 of NLRP3 were shown to essential for the NLRP3-Nek7 interaction in mice.<sup>54</sup> In a different study carried out in mouse and human macrophages, recruitment of NEK7 to NLRP3 was controlled by phosphorylation of Serine-803 (S803) in the LRR domain of NLRP3 (S806 in humans).<sup>55</sup> These findings suggest an important regulatory mechanism targetable by small molecules, to disrupt NLRP3 inflammasome assembly.

The majority of stimuli that activate the NLRP3 inflammasome do not directly bind to NLRP3<sup>56,57</sup> and mitochondria have been proposed as the missing link underlying this mechanism. Mitochondria can be damaged by danger signals, such as  $K^+$  efflux or changes in intracellular Ca<sup>2+</sup>, resulting in mitochondrial DNA (mtDNA) release into the cytosol, triggering reactive oxygen species (ROS) and oxidisation of the mtDNA, which may become an NLRP3 ligand.<sup>58,59</sup>In support of this model of ROS-modified mtDNA as an NLRP3 activator, macrophages lacking mtDNA show reduced IL-1 $\beta$  production<sup>58</sup>; however, questions remain about the high concentration of ROS inhibitors used, leading to potential artefacts or inhibition of NLRP3 priming. 43,60 Nevertheless, LPS triggers MvD88 and TRIF signalling, and activates IRF1-dependent transcription of CMPK2 (a mitochondrial nucleotide kinase) to enable mtDNA synthesis.<sup>61,62</sup> This newly synthesised mtDNA is particularly susceptible to oxidisation, and may release fragments of ox-mtDNA into the cytosol to co-localise with components of the NLRP3 inflammasome in macrophages.<sup>61</sup> This colocalisation of NLRP3 induced ASC-specks and oxmtDNA, however, is not observed with AIM2induced ASC specks.<sup>61</sup> Associations between circulating mtDNA and NLRP3 are also emerging. with potential as biomarkers of NLRP3 activation and increased levels of cell-free circulating mtDNA have been linked to a poor prognosis in patients with COVID-19.63 Indeed, coronaviruses have been shown to activate the NLRP3 inflammasome, via either indirect interaction with NLRP3 (SARS-CoV E viroporin protein, viroporin 3a) or direct activation with NLRP3 (SARS-CoV-2 N protein), resulting in hyperinflammation and increased COVID-19 severity.<sup>64-66</sup> Finally, guanylate-binding proteins (GBPs), members of the GTPase family, are associated in the regulation of several inflammasomes.<sup>67</sup> Increased levels GBPs have been reported in certain inflammatory and autoimmune disorders with important implications for the NLRP3.<sup>68</sup> In the context of NLRP3, human GBP5 binds the pyrin domain of the NLRP3 increasing complex formation.<sup>69</sup> Moreover, Gbp5<sup>-/-</sup> bone marrow-derived macrophages show impaired activation of the NLRP3 inflammasome, but not NLRC4, with low levels of IL-1 $\beta$  and IL-18.<sup>69</sup>

# NLRP 6 AND 7

The NLRP6 inflammasome has been implicated in a number of different physiological processes, including host defence against microbial infections,<sup>70-73</sup> maintenance of epithelial integrity<sup>73,74</sup> and regulation of neuroinflammation.<sup>75,76</sup> The initial characterisation of NLRP6 identified it as having a unique property among the NLRs in that it activates both caspase-1 and NF- $\kappa$ B,<sup>77</sup> although, interestingly, it has also been shown to negatively regulate NF- $\kappa$ B downstream of TLR signalling in a mouse model.<sup>72</sup>

Evidence strongly suggests that NLRP6 assembles an inflammasome. NLRP6 co-localises with ASC, both under steady-state conditions and following bacterial infection in vitro.<sup>70,77,78</sup> In vivo studies showed reduced serum IL-18 levels in  $NIrp6^{-/-}$  mice, both under steady-state conditions and following dextran sulphate sodium (DSS)induced colitis than in WT mice,<sup>79,80</sup> as well as reduced IL-1 $\beta$  levels in bronchoalveolar lavage fluid from  $NIrp6^{-/-}$  mice compared with WT following MRSA infection.<sup>70</sup> However, in these same studies, IL-1 $\beta$  expression was increased in the colon of NIrp6<sup>-/-</sup> mice<sup>79</sup> and both IL-1 $\beta$  levels and caspase-1 activity were comparable between Nlrp6<sup>-/-</sup> and WT mice following infection with various bacteria,<sup>72</sup> making it unclear whether NLRP6 inflammasome assembly occurs during systemic bacterial infection.

More recently, the NLRP6 inflammasome was shown to be activated by Gram-positive bacteria, including *Staphylococcus aureus* (MRSA) and *Listeria monocytogenes*, leading to IL-1 $\beta$  and IL-18 maturation and pyroptosis.<sup>70,71,81</sup> Lipoteichoic acid (LTA) has been implicated in the initial 'priming' signalling event; it has been found to upregulate NLRP6 and caspase-11 expression via type 1 interferon signalling,<sup>71</sup> as well as activating the NLRP6 inflammasome through the ASC-caspase-11-caspase-1 signalling pathway.<sup>71</sup>

The NLRP7 inflammasome is also incompletely characterised, with evidence suggesting that to recognises cell wall components and microbial acylated lipopeptides, and promotes ASCdependent activation of caspase-1, with IL-1 $\beta$  and IL-18 maturation to restrict intracellular bacterial replication.<sup>82</sup> Activation of this proposed inflammasome following Staphylococcus aureus infection has been suggested to require ATP binding at the NLRP7 Walker A motif,<sup>83</sup> as well as being regulated by NLRP7 ubiguitination.<sup>84</sup>

Although further study defining the NLRP7 inflammasome is required, there is also substantial evidence to support NLRP7 as a negative regulator of inflammasome signalling. Reduced IL-1ß release has been observed in NLRP7 overexpression studies in HEK293 cells77,85 and in peripheral blood mononuclear cells (PBMCs) from patients with disease-associated loss-of-function NLRP7 variants,<sup>86</sup> whereas NLRP7 mutants that resulted in truncated NLRP7 protein did not inhibit IL-1ß release.<sup>87,88</sup> Various mechanisms have been proposed for this regulation of IL-1 $\beta$ , including direct interaction with and inhibition of inflammasome components, and alteration of pro-IL-1ß transcription and subsequent trafficking and release of the mature forms,89 although further validation is required for this, as various reports have also shown a lack of effect of NLRP7 on inflammatory responses.77,82,84,90 Taken together, these studies illustrate a multifaceted role for the NLRP7 inflammasome in the regulation of inflammatory responses. Taken together, these studies illustrate a multifaceted role for the NLRP7 in the regulation of inflammatory responses, wherein it may negatively regulate the inflammasome in the resting state but assemble an inflammasome following a stimulus such as bacterial infection.

# **PYRIN**

Similar to NLRP3, the pyrin inflammasome detects intracellular pathogens and is tailored to counteract bacterial toxins from different species.<sup>91</sup> The human pyrin protein is composed of four main domains: an N-terminal PYD domain which interacts with ASC with subsequent activation of caspase-1: a B-box domain 60 to 280 amino acids long; a central helical scaffold (CHS) connected to the B30.2 domain on its C-terminus (Figure 1),<sup>92</sup> and the B30.2 domain, where the majority of pathogenic mutations are found in

humans, but this domain is absent in mice. The Pyrin inflammasome sensor detects inhibition of RhoA subfamily GTPases activity and is activated by various RhoA inhibiting toxins, including TcdA and TcdB from Clostridioides difficile (C. difficile) RhoA is a member of the GTPases superfamily that activates protein kinase N1 (PKN1) and PKN2, thereby facilitating binding of the inhibitory protein 14–3-3 to pyrin by phosphorylating pyrin at Ser208 and Ser242, which results in the inactive form of pyrin.<sup>91-93</sup> This inflammasome is triggered when bacterial toxins, such as C. difficile toxin A or B,<sup>91</sup> inactivate RhoA, reducing PKN1 and PKN2 activity, dissociating 14-3-3 from pyrin<sup>93</sup> and fully activating pyrin, to culminate in caspase-1 dimerisation and cleavage to caspase-1 and production of mature forms of IL-18 and IL-18.94 Aberrant actin depolymerisation can activate the pyrin inflammasome inducing autoinflammation driven by IL-18, but not IL-1 $\beta$ .<sup>95</sup> Mice homozygous for a hypomorphic allele of Wdr1 manifested several cutaneous features which were similar to those presented by patients with autoinflammatory disorders. Finally, this unique pyrin inflammasome activation, driven by aberrant actin depolymerisation, was only observed in monocytes and not in macrophages or neutrophils.

Several autoinflammatory disorders have been linked, directly or indirectly, to the pyrin inflammasome<sup>14,96</sup> and will be discussed later (Figure 3). Mutations in the MEFV gene, which encodes pyrin in humans, are known to cause FMF.<sup>97</sup> Receptor-interacting serine/threonineprotein kinase 3 (RIPK3) was recently shown to influence pyrin activation by exerting an inhibitory effect on the mammalian target of rapamycin (mTOR), leading to transcription of MEFV and pyrin inflammasome activation.<sup>98</sup> Another study has demonstrated that while pyrin dephosphorylation was not sufficient to induce its activation in healthy individuals, the opposite was the case in FMF patients, with inflammasome activation, IL-1 $\beta$  secretion and pyroptosis in monocytes.<sup>99</sup> These molecular insights are of great importance in several autoinflammatory disorders and may form basis of new treatments for patients harbouring disease-causing mutations. Activation of the pyrin inflammasome can also be induced by two microbiome-derived bile acid metabolites, BAA485 and BAA473, revealing an unexplored role of pyrin in the lower gastrointestinal tract.<sup>100</sup> Finally, while many MEFV mutations cause FMF, a recent study also showed



**Figure 3.** Inflammasome sensors, activators and related disorders. The NLRP1, NLRP3, pyrin, NLRC4 and AIM2/IF16 with their current ligands and intracellular mediators involved in activation of these molecules. NLRP1 detects lethal toxin *Bacillus anthracis* (*B. anthracis*), *Shigella flexneri* (*S. flexneri*), *Listeria monocytogenes* (*L. monocytogenes*). NLRP3 is an overall sensor for several PAMPs and DAMPs (Figure 2) responding to intracellular damage induced by pathogenic or sterile insults. Pyrin inflammasome is activated by bacterial toxins that modify RhoA GTPases. The NLRC4 inflammasome detects bacterial proteins via NLR family-apoptosis inhibitory proteins (NAIPs) and can assemble an inflammasome with or without ASC. Absent in melanoma 2 (AIM2) and IFN<sub>Y</sub>inducible protein-16 (IFI16) detect dsDNA via their HIN-200 domains. When these sensors are chronically activated or not properly regulated, inflammatory-related conditions are caused by these inflammasomes. MSPC, Multiple self-healing palmoplantar carcinoma; FKLC, Familial keratosis lichenoides chronica; NAIAD, NLRP1-associated autoinflammation with arthritis and dyskeratosis; MWS, Muckle–Wells syndrome; FCAS, familial cold autoinflammatory syndrome; MDS, myelodysplastic syndromes; neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurological nutaneous and articular (CINCA); PAAND, pyrin associated autoinflammation with neutrophilic dermatosis; FMF, familial mediterranean fever; PAPA, pyogenic arthritis, pyoderma gangrenosum, and acne; MKD, mevalonate kinase deficiency; AIFEC, autoinflammation and infantile enterocolitis; NLRC4-MAS, NLRC4 macrophage activation syndrome; CKD, chronic kidney disease.

that certain MEFV mutations, such as MEFV\_p.M680I, confer protection against Y. pestis infection by inducing high levels of IL-1B. Therefore, it is proposed that perhaps Y. pestis influenced evolutionary selection of some FMFassociated mutations in certain human populations.<sup>101</sup>

#### NAIP-NLRC4

The NLR family CARD domain-containing 4 (NLRC4) inflammasome is activated by bacterial flagellin and T3SS secretion system rod proteins and forms an integral component of the immune response against cytoplasmic Gram-negative bacteria.<sup>102</sup> In contrast to other inflammasomes, regulation by the NLRC4 inflammasome is dependent on its cooperative activity with NLR apoptosis inhibitory proteins (NAIPs), an NLR subfamily of proteins that

act as cytosolic innate immune receptors for specific bacterial ligands. Whilst the mouse genome encodes NAIP1, NAIP2 and NAIP5/6, 103-106 there is only one human NAIP and this recognises the T3SS needle protein.<sup>107</sup> Following detection of the relevant ligands, NAIPs bind to NLRC4 to induce a structural reorganisation from its autoinhibited state to its active configuration,<sup>108</sup> which in turn recruits caspase-1<sup>109</sup>; NLRC4 can bind to caspase-1 directly via its CARD domain or indirectly via the adaptor molecule ASC that functions as an intermediary between NLRC4 and caspase-1.109 Caspase-1 activation leads to IL-1 $\beta$  and IL-18 release, with IL-1 $\beta$  stimulating the recruitment of neutrophils to destroy extracellular bacteria. Caspase-1 also initiates removal of intracellular bacteria via pyroptotic cell death, releasing the bacteria and exposing them to uptake and killing by ROS in neutrophils.<sup>110,111</sup>

Pyroptotic bacterial clearance has led to further investigation of the role of NLRC4 in PANoptosis, inflammatory programmed cell an death mechanism controlled by a multimeric protein called the PANoptosome. This large complex comprises molecules from the programmed cell death pathways of pyroptosis, apoptosis and necroptosis,<sup>112</sup> and NLRC4 has been implicated in caspase-mediated cleavage of the DNA damage sensor poly (ADP-ribose) polymerase 1 (PARP1), a hallmark of apoptosis.<sup>113</sup> Furthermore, Salmonella typhimurium (S. typhimurium) infection induces both NLRC4 inflammasome activation and alongside other forms of cell death in macrophages.<sup>114</sup> S. typhimurium clearance is also dependent on either caspase-8 driven apoptotic, pyroptotic or necroptotic pathways, demonstrating cellular flexibility to protect against intracellular infections.<sup>115</sup> As long as caspase-1 or caspase-8 are S. typhimurium activated during infection, macrophages can undergo a form of cell death to control S. typhimurium infection, which can be driven by the NLRC4 or other unknown factors.<sup>115</sup>

Four clinical phenotypes of NLRC4 inflammasome-related diseases, because of gainof-function NLRC4 mutations, have been reported; autoinflammation and infantile enterocolitis (AIFEC), NLRC4 macrophage activation syndrome (NLRC4-MAS), familial cold autoinflammatory syndrome (FCAS) and chronic infantile neurological cutaneous and articular (CINCA), also known as neonatal-onset multisystem inflammatory disease (NOMID).<sup>116</sup> However, NOMID/CINCA is often more associated with NLRP3 mutations. The severity of symptoms range from AIFEC and NLRC4-MAS, which require frequent hospitalisations for lifethreatening episodes, to FCAS4 with milder phenotypes of urticarial rash and arthritis.<sup>117,118</sup> However, progress in this field is slow as there are relatively few known patients with pathogenic NLRC4 mutations.<sup>119</sup>

# AIM2

Absent in Melanoma 2 (AIM2) is a cytosolic sensor that recognises double-stranded DNA, binding through its C-terminal domain HIN-200 domain, and resulting in AIM2 inflammasome activation.<sup>120</sup> Ongoing research shows that AIM2 is multifunctional, acting as a host defence against a range of pathogens, and also been implicated in a multitude of diseases.<sup>120,121</sup> Like the NLRP3 inflammasome, the AIM2 inflammasome pathway

involves AIM2 activation and oligomerisation. ASC binding (through AIM2 and ASC PYDs), caspase-1 dependent cleavage and activation of IL-1 $\beta$ , IL-18 and GSDMD, and subsequent pyroptosis.<sup>121</sup> The assembly of AIM2-ASC has also been implicated in caspase-8 and downstream caspase-9 activation causing caspase-3 dependent, caspase-1 independent, apoptosis.<sup>122</sup> AIM2 inflammasome signalling has been shown to play a key role in neurodevelopment and CNS homeostasis, through its role in GSDMD regulation.<sup>123</sup> AIM2 also has a role in early brain injury, following subarachnoid haemorrhage, as patients had significantly increased cerebral spinal fluid (CSF) levels of AIM2, and neuronal damage was reversed in AIM2 and caspase-1 KO models.<sup>124</sup> An additional role of AIM2 has emerged, linked to a regulatory role of pyrin and ZBP1, with the ability to induce PANoptosis through multi-protein interactions of AIM2, pyrin, ZBP1, ASC, caspase-1 and -8, RIPK-3 and -1 and FADD through pyroptosis, apoptosis and necroptosis pathway interactions, following herpes simplex virus 1 (HSV-1) and Francisella novicida (F. novicida) infection.<sup>125</sup> Despite AIM2's major role in cytosolic DNA detection and inflammasome activation, this is dispensable in human myeloid cells; instead, the cGAS-STING pathway is the main perpetrator of lysosomal cell death, by inducing NLRP3 activation, resulting from upstream  $K^+$  efflux and initiating antiviral immunity.<sup>126</sup> Moreover, AIM2 senses radiationinduced DNA damage, in intestinal epithelial and bone marrow cells, thereby activating the inflammasome with subsequent cellular death.<sup>127</sup> This has therapeutic implications for AIM2 manipulation and control of inflammation. Recently, AIM2 was shown to play an important role in driving aldosterone-induced renal injury via ER stress, with important implications in the treatment of chronic kidney disease (CKD).<sup>128</sup>

High-mobility group box-1 (HMGB1) has been AIM2-ASC implicated in inflammasome activation.<sup>129</sup> Circular RNA PPP1CC was recently shown to stimulate Porphyromonas gingivalis LPSinduced pyroptosis in vascular smooth muscle cells, by AIM2 activation through the HMGB1/ TLR9/AIM2 pathway, also promoting atherosclerosis.<sup>130</sup> This study reported in vitro siRNA knockdown of circPPP1CC in HUVEC cells, resulting in pyroptosis decrease and reduced levels of HMGB1, TLR9, AIM2 and caspase-1. HMGB1 has been implicated in LPS-induced acute lung inflammation by activating the TLR2, TLR4

and RAGE/NF- $\kappa B$  pathways in mice, with AIM2 inflammasome activation and M1 macrophage polarisation.  $^{131}$ 

GBP2 and GBP5 are important promoters of AIM2 inflammasome activation by inducing cytosolic bacteriolysis and DNA release during *F. novicida* infection.<sup>132</sup> *F. novicida* covered with GBPs is targeted by immunity-related GTPase B10 (IRGB10) with further activation of AIM2.<sup>133</sup> IRGB10 was also shown to contribute towards activation of the caspase-11–NLRP3 inflammasome in response to Gram-negative bacteria, other than *F. novicida*.<sup>133</sup>

# IFI16

In a similar manner to AIM2, IFN $\gamma$ -inducible protein-16 (IFI16) is an intracellular innate sensor that detects viral RNA, single and double-stranded viral DNA.<sup>134-137</sup> AIM2 and IFI16 are members of the pyrin and HIN domain (PYHIN) protein family, and these two are also known as ALRs.<sup>135,138</sup> IFI16 has been shown to detect herpes simplex virus type 1 (HSV-1),<sup>135</sup> Kaposi sarcoma-associated herpesvirus,<sup>139</sup> vaccinia virus,<sup>140</sup> HIV,<sup>134</sup> chikungunya virus<sup>141</sup> and influenza A virus.<sup>136</sup> Unlike AIM2, IFI16 contains two HIN-200 subunits, HINa and HINb, that detect the nucleic acid in question,<sup>135</sup> or enhance the transcription of retinoic acid-inducible gene-I (RIG-I).<sup>136,137</sup>

Activation of IFI16 through sensing nucleic acids induces activation of the STING-TBK1 pathway, which leads either to the induction of type I IFNs through IRF3,<sup>142</sup> IL-1 $\beta$  secretion by interactions of its pyrin domain that interacts with caspase-1 and ASC to form a functional inflammasome, <sup>139</sup> or NF- $\kappa B$  activation after nuclear DNA damage.<sup>143</sup> IFI16 has also been associated with cervical cancer progression by inducing the immune checkpoint programmed cell death 1 ligand 1 (PD-L1) via the STING/TBK1/NF-KB pathway.<sup>144</sup> This could be of hiah importance because of the immunomodulatory effects of PD-L1 in several cancers.<sup>145,146</sup> Regarding inflammatory-related disorders, the serum levels of IFI16, as well as anti-IFI16 antibodies, were elevated in psoriatic arthritis (PsA), which was more pronounced in patients with high CRP levels.<sup>147</sup> Moreover, STING also negatively regulates IFI16 by recruiting the E3 ligase TRIM21, which degrades IFI16 through ubiquitin-mediated proteasomal degradation, thus preventing IFN-I overproduction.<sup>148</sup> IFN regulation is a fundamental cellular mechanism to avoid autoinflammation, and a transcript isoform of IFI16 (IFI16- $\beta$ ), inhibits the formation of the AIM2-ASC complex.<sup>149</sup> IFI16 is a key sensor of viral nucleic acids, in regulating IFN and modulating the inflammatory response.

# **COPS AND POPS**

CARD-only proteins (COPs) and pyrin-only proteins (POPs) are small, cytoplasmic endogenous regulators of inflammasome assembly which are present in humans but absent in the mouse and rat genome.<sup>150</sup>

Pyrin-only proteins consist of the PYD alone and are named POP1, POP2, POP3 and POP4. Expression of POP1 and POP2 is induced by several hours of TLR activation, 151, 152 following which they interact with the PYD of ASC.<sup>153-155</sup> seemingly binding competitively with this domain to prevent ASC oligomerisation, IL-1 $\beta$  and IL-18 release and pyroptosis.<sup>152,156-158</sup> Silencing of POP1 in human macrophages is reported to exacerbate inflammation, whereas POP1 expression had a robust anti-inflammatory effect in mice.<sup>151</sup> POP2 been shown to inhibit additionally has NF- $\kappa$ B<sup>152,154,155,158</sup>; however, elevated IFN $\gamma$  levels were seen in POP2-expressing transgenic mice, suggesting a mechanism by which POP2 heightens immune responses.<sup>158</sup> POP3 differs in that it binds to the PYDs of the DNA sensors AIM2 and IFI16, but not of ASC, 159 and is stimulated by dsDNA viruses.<sup>159</sup> POP3 appears not to affect NF- $\kappa$ B and MAPK signalling or inflammatory cytokine release in response to NLR inflammasome agonists, but instead selectively inhibit AIM2-like receptor (ALR) inflammasomes and their downstream inflammatory processes.<sup>159</sup> Finally, POP4 is the most recently discovered POP whose expression is upregulated by LPS stimulation.<sup>160</sup> It inhibits NF- $\kappa$ B activity following TNF stimulation by blocking TLR-induced ReIA/p65 transactivation and secretion of NF- $\kappa$ B-regulated cytokines, such as TNF and IL-6.<sup>160</sup>

CARD-only proteins, in contrast, consist of a CARD domain and comprise CARD16 (pseudo-IL-1 $\beta$  converting enzyme (pseudo-ICE)/Cop), CARD17 (Inca), and CARD18 (Iceberg). The first of these, CARD16, binds competitively with the caspase-1 CARD, preventing its dimerisation and reducing the maturation and release of IL-1 $\beta$ .<sup>161-163</sup> It also interacts with the ASC CARD<sup>164</sup> and may affect NF- $\kappa$ B activation via the CARD-containing kinase receptor-interacting-serine/threonine-protein

kinase 2 (RIP2).<sup>161,162</sup> Nevertheless, these studies were mainly carried out in cell lines in which CARD16 was transiently overexpressed,<sup>164</sup> further investigation in primary cells is required to support the physiological relevance of these studies. Similarly, CARD17 interacts with itself to form dimers, also binding to other COPs and the CARD of caspase-1.<sup>162,165</sup> Its expression is upregulated by pro-inflammatory stimuli, in particular IFN $\gamma$ .<sup>162</sup> The proposed mechanism of action of CARD17 is that it binds specifically to the filamentous form of caspase-1, localising at the tip of caspase-1 CARD filaments to act as a cap, thereby preventing caspase-1 CARD polymerisation and subsequent inflammasome activity.<sup>165</sup> In contrast to CARD16, CARD17 does not affect TNF-induced NF-κB activation, 162, 165 apparently specifically targeting caspase-1 activation. CARD18 interacts with the pro-caspase-1 CARD, well as as selfaggregating, <sup>161,165,166</sup> although the evidence regarding its function is conflicting. CARD18 has been implicated as acting in a negative feedback mechanism to inhibit RIP2 binding to caspase-1 and disrupting existing RIP2/caspase-1 complex to reduce IL-1 $\beta$  release.<sup>161,166</sup> However, one study found that CARD18 did not inhibit inflammasome activation, despite being incorporated into caspase-1.<sup>165</sup> This may be because CARD18 expression is upregulated by LPS or TNF stimulation over several hours, suggesting this protein is produced slowly so that the beneficial effects of inflammation can occur before the process is halted.<sup>166</sup> Further studies in relevant cellular models are required to determine the role of this protein.

# NON-CANONICAL INFLAMMASOME

The one-step non-canonical inflammasome senses the cytosolic presence of LPS derived from Gramnegative bacteria, specifically by monocytes.<sup>167</sup> LPS bind directly to caspase-4 (caspase-11 in mice) and caspase-5, which initiates NLRP3-caspase-1dependent IL-18 and IL-1<sup>β</sup> cleavage and secretion, as well as driving inflammatory cell death via pyroptosis.<sup>168,169</sup> GSDMD-dependent This mechanism of inflammasome activation was first reported by Kayagaki et al. in 2011 using genetic depletion of caspase-11 in C57BL/6 mice.<sup>2</sup> The presence of a mutation in caspase-11 exon 5 in mice, resulted in failure to induce macrophage cell death and the absence of IL-18 and IL-1 $\beta$  in the serum upon E. coli, C. rodentium and

*V. cholerae* infection. This study also elucidated the role of caspase-11 in the induction of lethal septic shock in mice.

of Aberrant activation non-canonical inflammasome factors has been associated with a range inflammatory pathologies; for instance, a case of a 10-year-old girl diagnosed with Aicardi-Goutières syndrome positive (AGS), for heterozygous IFIH1 mutation (c.2336G > A (p.R779H)). The patient showed elevated histological levels of caspase-4 and caspase-5 processing in intestinal tissue.<sup>170</sup> This was correlated with symptoms such as diarrhoea, inflammatory cell infiltration and increased levels of IL-6 and IFN $\alpha$  in serum, underlining the importance of the non-canonical inflammasome in clinical development of this case.

A Finnish family, presenting a gain-of-function mutation (Arg219His) in the late myeloid differentiation factor CCAAT enhancer-binding protein epsilon (C/EBPe), developed an autosomal recessive neutrophil-specific granule deficiency.<sup>171</sup> Clinically, these patients present symptoms related with autoinflammation, immunodeficiency and neutrophil failure. Whole-transcriptome and gPCR screening of patients' granulocytes showed upregulation of PRTN3, a regulator of both IL-1 $\beta$ and IL-18. Canonical (NLRP3, IL-18) and noncanonical inflammasome elements (CASP5) were transcriptionally upregulated in macrophages.<sup>171</sup> Indeed, histological examination of LPS-stimulated macrophages derived from these patients revealed a unique signal of caspase-5 but not caspase-1 cleavage, compared with healthy controls. This study supports the functional relevance of the non-canonical inflammasome in autoinflammatory diseases.

Other studies have highlighted an important role of the non-canonical inflammasome in senescence biology. The senescence-associated secretory phenotype (SASP) is partly controlled by activation of the non-canonical inflammasome in vitro.<sup>172</sup> Genetic depletion of caspase-5 reduced the secretion of hallmark cytokine factors related to SASP, such as of IL-1 $\alpha$ , IL-6, IL-8 and MCP-1 factors in fibroblasts.<sup>173</sup> In a different study, augmented expression levels and activation of caspase-4, via cvtosolic LPS, induced a senescence state and pyroptotic cell death in IMR-90 fibroblasts.<sup>174</sup> This was reflected by raised levels of p16INK4a, p21CIP1 proteins, and increased activity of senescenceassociated- $\beta$ -galactosidase. Moreover, in vivo studies using NF- $\kappa$ B KO mice showed a correlation

in the expression levels of caspase-11 with p21 in lung epithelial cells. Finally, caspase-11 expression also correlated with loci of telomere-associated DNA-damage response, a hallmark of senescence, in alveolar cells of 24-month aged mice. Overall, these studies support the notion that induction of non-canonical inflammasome pathways is relevant to the biology of senescence and may present a viable therapeutic opportunity for a variety agerelated diseases.

#### INFLAMMASOMES IN AUTOINFLAMMATORY DISORDERS

#### TRAPS

TRAPS is a rare monogenic autosomal dominant autoinflammatory disorder linked to mutations in TNFRSF1A.<sup>175</sup> Its clinical manifestations include prolonged bouts of fever, associated with marked left-shifted leukocytosis, skin rash, per-orbital oedema. pleuritic chest pain and AA amyloidosis.<sup>176,177</sup> To date, over 100 pathogenic variants of TNFRSF1A have been reported in Infevers,<sup>178</sup> and 97 of these are single-nucleotide missense mutations within exons 2, 3 and 4, and 80% of the variants are located within the first two cysteine-rich domains (CRD1, CRD2).

Our understanding of TRAPS pathophysiology has been hindered by the marked heterogeneity between each variant, and varving experimental conditions, such as differences in cell lines, mutant constructs for transfection and cell-type effects.<sup>179</sup> Blood soluble TNFR1 levels are reduced by about 50% in people affected by TRAPS, and this information was paramount to gene discovery.<sup>175</sup> Thus, an initial hypothesis was that impaired shedding of the receptor TNFR1 resulted in reduced levels of circulating TNFR1 acting as a TNF inhibitor.<sup>175</sup> But shedding anomalies could not be reproduced among all TNFR1 variants and all cell types, 179 and further data showed that mutated TNFR1 subcellular traffic was disturbed, leading to abnormal ER retention and reduced cellular membrane expression.<sup>179,180</sup> Mutated ERretained TNFR1 self-aggregates<sup>180</sup> and probably transduces inflammatory signals on its own,<sup>179-181</sup> as TRAPS patients' monocytes show ligandindependent NF- $\kappa$ B activation,<sup>179</sup> particularly as mutated TNFR1 is often unable to bind TNF.<sup>181</sup> TNFR1 lead variants also to increased mitochondrial ROS (mtROS) production, a known inflammasome.<sup>182</sup> activator of the NLRP3

Inhibition of mtROS impedes pro-inflammatory cytokine production in mouse embryonic fibroblasts transfected with TNFRSF1A pathogenic variants.<sup>182</sup> Mutated variants of TNFR1 also induce atypical ER stress and unfolded protein response (UPR) transcriptomic signature.<sup>183</sup> This is likely to result in NLRP3 activation, as ER-retained TNFR1 can recruit the ER stress sensor, IRE1a, an essential UPR effector<sup>184</sup> whose inhibition prevents NLRP3 activation.<sup>185,186</sup> Indeed, IRE1 $\alpha$  can promote NLRP3 inflammasome assembly bv damaging mitochondria via caspase-2 and Bid activation.<sup>185</sup>

These molecular elements correlate quite well with therapeutics. Indeed, TNF blockade is TRAPS,<sup>187</sup> inconsistently efficient in as endocellular TNFR1 aberrantly signals independently of its ligand. Meanwhile, IL-1 blockade is very effective in producing clinical remission in selected patients, 177, 188 which suggests that the NLRP3 inflammasome is proinflammatory responsible for cvtokine secretion in TRAPS. If confirmed, NLRP3 inhibitors, such as MCC950<sup>189</sup> and OLT1177,<sup>190</sup> could be candidates for tailored treatment in TRAPS.

#### CAPS/NLRP3-AIDs

Cryopyrin-associated periodic syndromes also known as NLRP3-associated autoinflammatory diseases (NLRP3-AIDs) comprise a series of rare monogenic autoinflammatory conditions which range in severity from the mildest FCAS, to the moderate Muckle-Wells syndrome (MWS), and more severe CINCA/NOMID.<sup>191</sup> These disorders are caused by gain-of-function mutations in the NLRP3 gene, with almost 100 known pathogenic mutations.<sup>192</sup> CAPS-related mutations are associated with systemic inflammation, which may lead to irreversible organ damage if untreated, and symptoms including urticaria-like rash, coldinduced fever and sensorineural hearing loss.<sup>191</sup>

The pathogenesis of these disorders stems from the constitutive activation of the NLRP3 inflammasome and associated increase in caspase-1 activity, IL-1 $\beta$  and IL-18 release and pyroptosis.<sup>193,194</sup> Monocytes and macrophages from CAPS patients release a constitutively high level of IL-1 $\beta$  compared with healthy controls,<sup>195-197</sup> with IL-1 $\beta$  secretion occurring much faster in monocytes from CAPS patients.<sup>198</sup> The NLRP3 inflammasome in monocytes from FCAS patients can also be activated by mildly hypothermic temperatures of 32°C.<sup>199</sup> Monocytes from CAPS patients and DCs from mice harbouring CAPS-related mutations also produce elevated IL-18. and mouse models of CAPS have shown that IL-1ß and IL-18 drive the pathology at different disease with IL-18 contributing stages, to early inflammation and IL-1<sup>B</sup> to later systemic inflammation.<sup>200</sup> Furthermore, significant inflammation was still observed when these mice were bred onto an IL-1 receptor (IL-1R) and IL-18 receptor (IL-18R)-deficient background, indicating that inflammation is not fully dependent on these cytokines in mice. In contrast, caspase-1 and GSDMD are both required for CAPS pathogenesis in mice, as knockout of each of these proteins prevented autoinflammatory symptoms in murine models.<sup>197,200-202</sup> Although further research is required in human studies, at this stage, it would seem that CAPS pathogenesis is primarily dependent on caspase-1 and GSDMD, and on hypersecretion IL-1 $\beta$  and IL-18 to a lesser extent.

The three IL-1 inhibitors currently used in clinical practice to treat CAPS are anakinra (Kineret), a recombinant version of the human IL-1R antagonist protein (IL-1Ra) canakinumab (Ilaris), a human monoclonal antibody which targets IL-1B, and rilonacept (Arcalyst), a fusion of the human IL-1R component (IL-1R1) and IL-1R accessory-protein (IL-1RAcP) which potently binds IL-1 $\beta$  and IL-1 $\alpha$ .<sup>203</sup> Potential future therapeutics are also emerging, including two small-molecule NLRP3 inhibitors related to the well-characterised MCC950,<sup>204</sup> Inzomelid (clinical trial identifiers NCT04015076 and NCT04086602) and Somalix. These inhibitors have completed phase 1 trials, with Inzomelid completing phase 1b in CAPS patients.<sup>205,206</sup> Further potential treatments which have completed phase 1 clinical trials include the tryptophan analogue, Tranilast (NCT03923140), a novel hybrid Fc-fused IL-1Ra (NCT02175056),<sup>207</sup> and ATI-450, a small-molecule inhibitor of the p38a mitogen-activated protein kinase (MAPK)/ MAPK-activated protein kinase 2 (MK2) inflammatory signalling pathway, 208 although the COVID-19 pandemic has hindered recruitment for phase 2 trials.

### **FMF and PAAND**

FMF is a monogenic autosomal recessive autoinflammatory disorder that was first described clinically in the late 1940s.<sup>209,210</sup> Patients with FMF suffer from recurring bouts of fever, abdominal or chest pain, arthritis, pseudo-erysipelas and they may also be afflicted with AA amyloidosis and mesothelioma. The disease was linked, in 1997, to mutations in the MEFV gene corresponding to the pyrin protein<sup>211,212</sup> (Figure 3).

Mutations in patients with FMF generally involve the C-terminal B30.2 domain of pyrin. This domain does not exist in rodents, imposing a significant caveat on the extrapolation of data from murine models.<sup>213</sup> Mutations in FMF do not impede the interaction of pyrin with caspase-1 and pro-IL-1 $\beta^{214}$ ; however, they substantially decrease the binding of pyrin to protein kinases N (PKN) 1 and 2.93 PKNs are kinases belonging to the protein kinase C superfamily and also RhoA GTPase effectors. These enzymes phosphorylate pyrin on serines-208 and -242, which enables 14-3-3 protein to bind and inhibit pyrin activation.<sup>93</sup> Thus, inactivation of RhoA triggers inflammation by removing pyrin inhibition by PKN-dependent phosphorylation and FMF mutations may decrease PKN-mediated inhibition of pyrin.<sup>93</sup>

Colchicine, which inhibits microtubular polymerisation,<sup>214</sup> remains the cornerstone of FMF treatment since the 1970s.<sup>215</sup> In healthy monocytes, pyrin activation depends on both serines-208 and -242 dephosphorylation and efficient microtubule assembly.<sup>99,216</sup> Colchicine inhibits this latter step without affecting the former; however, colchicine is less effective *in vitro* as an inhibitor of dephosphorylation-dependent pyrin activation in FMF monocytes than that in HC monocytes.<sup>99</sup>

Of note, an autosomal dominant disorder termed pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), clinically different from FMF, is caused by specific mutations in pyrin that abolish the 14–3-3 binding motif.<sup>217,218</sup> Interestingly, FMF mutations do not seem to affect 14–3-3 binding.<sup>217</sup> Serum levels of proinflammatory cytokines may be tenfold higher in FMF than in PAAND,<sup>99,217,218</sup> implying that additional control mechanisms, distinct from 14–3-3 binding, may be intact in PAAND but absent in FMF.

Mutations in *GSDMA*, *Gsdma3*, *GSDMB* and *GSDME* genes are associated with asthma, systemic sclerosis, alopecia, hearing loss and cancer<sup>219</sup>; while mutations in *GSDMD* are generally associated with inflammation in certain non-infectious conditions, normally driven by pyroptosis.<sup>219</sup> GSDMD has been linked with several non-infectious human diseases, such as autoimmune encephalomyelitis, Kawasaki disease, kidney disease, FMF and NOMID/CINCA where inflammation plays a crucial role in the

pathophysiology of the disease.<sup>219-221</sup> Ablation of *Gsdmd* in FMF and NOMID/CINCA mouse models protected the animals from the inflammatory-related symptoms normally presented during these conditions.<sup>220,221</sup>

Anti-IL-1 inhibitors acting downstream of inflammasome activation, are a second-line treatment for FMF,<sup>215,222</sup> and the treatment of choice in PAAND.<sup>14,217</sup> Anti-TNF inhibitors could be useful in certain patients with articular phenotypes, while small-molecule caspase-1 inhibitors could theoretically target FMF autoinflammation.

#### **FINAL REMARKS**

Molecular characterisation of inflammasomes has revolutionised our understanding of several immune-mediated inflammatory disorders. It is clear that inflammasomes are key protectors of tissues from pathogens, but, nevertheless, when these mechanisms are dysregulated, this can lead autoinflammation. Inflammasome to activitv requires tight regulation to avoid chronic inflammation. and. as seen in several autoinflammatory disorders. smallest the modification in protein composition may lead to autoinflammation. Understating these processes has led us to the development of several inhibitors which are essential to more effective therapeutics. Although our understanding of the intrinsic mechanisms underlying these disorders has grown exponentially, several questions remain regarding the pathogenesis of these disorders.

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### **CONFLICT OF INTEREST**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### **AUTHOR CONTRIBUTIONS**

Samuel Lara-Reyna: Conceptualization; funding acquisition; visualization; writing – original draft; writing – review and

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# **Graphical Abstract**

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This review explores the molecular processes underlying the activation of several inflammasomes and highlights their role during health and disease. We describe the harmful effects of these molecular complexes, in some autoinflammatory disorders, and review current therapeutic approaches as well as future prospective treatments.