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A dRAsTic RHOAdblock of Pyrin inflammasome activation

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Abstract

The Pyrin inflammasome guard is disabled if the activity of small cellular GTPase is compromised, in response to defects in the mevalonate pathway and directly by mutations in Pyrin, resulting in the IL-1 β -driven autoinflammatory diseases MKD and FMF.

Recognition of microbial ligands by pattern-recognition receptors (PRRs) triggers the formation of a cytosolic protein platform referred to as the inflammasome. During inflammasome assembly, the adaptor protein ASC and the protease caspase-1 are recruited to PRRs, followed by caspase-1 activation then processing and release of the pro-inflammatory cytokines IL-1 β and IL-18 and induction of pyroptotic cell death. While inflammasome formation is instrumental for host defense, it is detrimental if dysregulated, as is the case in human autoinflammatory diseases, including Cryopyrinopathy and familial Mediterranean Fever (FMF)¹. Several PRRs have been described to assemble an inflammasome, including Nod-like receptors (NLRs), AIM2-like receptors (ALRs) and Pyrin. While the mechanism and function of NLR and ALR inflammasomes are quite well understood, the precise role of Pyrin in inflammasome signaling has been controversial. Initially, contradicting studies suggested Pyrin either promoted or inhibited inflammasome activation and potential Pyrin ligands had been elusive, until a distinct mode of operation for the Pyrin inflammasome was uncovered. It's now known that Pyrin senses modification and inactivation of Rho family GTPases by bacterial toxins, which promotes the assembly and activation of a Pyrin inflammasome² (Fig. 1a). Two papers in this issue of *Nature Immunology* by Chae and colleagues³ and Wang and colleagues⁴ now provide exciting insights into the mechanism of Pyrin inflammasome regulation by small cellular GTPases through geranylgeranylation, a post-translational lipid modification. Thus, defects in the fundamental metabolic mevalonate kinase (MVK) pathway that synthesizes geranylgeranyl pyrophosphate, the substrate of geranylgeranylation, lead to Pyrin inflammasome activation. Therefore, the two seemingly unrelated autoinflammatory diseases FMF - caused by a mutation in Pyrin, and mevalonate kinase (MVK) deficiency (MKD) (also known as

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hyperimmunoglobulinemia D syndrome, HIDS) - caused by a loss-of-function mutation in MVK, both share a common mechanism – Pyrin inflammasome activation.

MEFV, which encodes Pyrin, was the first inflammasome gene linked to a human disease, namely FMF. Its N-terminal protein binding domain, which has a key role in inflammasome assembly, is now referred to as the PYRIN domain (PYD) and is present in many inflammasome components⁵. Due to its recessive mode of inheritance, FMF was originally considered to be a disease caused by loss of function mutations - a view that has since been challenged¹. In addition to its role as an inflammasome sensor, Pyrin also interacts with Caspase-1 through its B30.2/SPRY domain to modulate IL-1 β release and is itself cleaved by Caspase-1, resulting in a truncated Pyrin that activates NF- κ B, which may further amplify autoinflammatory responses since FMF-linked mutations are cleaved more efficiently. However, these mouse studies were complicated by the fact that mouse Pyrin lacks the B30.2/SPRY domain, which contains the majority of FMF-linked mutations¹. In contrast, MKD is an autosomal recessive autoinflammatory disease, which also shows a wide clinical spectrum, dependent on the level of MVK activity. MVK-mediated geranylgeranylation promotes IL-1 β release⁶, but the underlying mechanism has been elusive.

Wang and colleagues investigated the pathology of MKD⁴. The fundamental metabolic mevalonate pathway is responsible for cholesterol biosynthesis and other metabolites, whereby MVK-mediated phosphorylation of mevalonate is a key step and MKD causes HIDS⁷. Geranylgeranylation of small GTPases, which is facilitated by the geranylgeranyl transferase I (GGTase I), functions, at least in part, as a membrane anchor. The authors show that geranylgeranylation enables Toll-like receptor (TLR)-induced PI3K activation. *Pggt1b*^{-/-} (which encodes GGTase I) macrophages show elevated pro-inflammatory cytokines and *Pggt1b*^{-/-} mice are highly susceptible to lipopolysaccharide-induced septic shock. The cytokine profile of *Pggt1b*^{-/-} mice is similar to mice deficient in PI3K or Akt1, and *Pggt1b*^{-/-} macrophages display impaired Akt1 activation and phenocopy *Pik3cd*^{-/-} (which encodes the PI3K catalytic p110 δ subunit) macrophages. Ras GTPases directly bind to- and activate PI3K catalytic subunits, but an siRNA screen by Wang and colleagues specifically identifies Kras as being necessary for Akt1 activation and they find Kras geranylgeranylation essential for its binding and activation of p110 δ . *Pggt1b*^{-/-} and *Pik3cd*^{-/-} macrophages exhibit constitutive IL-1 β release, dependent on Pyrin, ASC and Caspase-1, but independent of NLRP3, AIM2 or NLRC4, demonstrating that specifically the Pyrin inflammasome is activated. Both, *Pggt1b*^{-/-} and *Pik3cd*^{-/-} macrophages, display higher basal and inducible expression of *Mefv*. Congruently, HIDS patients displayed higher expression of pro-inflammatory cytokines, reminiscent to healthy peripheral blood mononuclear cells treated with statins, which also block the mevalonate pathway. Hence, the authors concluded that in the absence of protein geranylgeranylation, PI3K activity is compromised and therefore promotes unchecked TLR-induced inflammatory responses causing the constitutive activation of the Pyrin inflammasome and IL-1 β release (Fig. 1b). Hence, GGTase I is able to control the activation of the Pyrin inflammasome through a PI3K-p110 δ -dependent pro-inflammatory transcriptional response.

In the related paper, Chae and colleagues followed up on an earlier report and investigate the mechanism, by which bacterial toxins that inactivate Rho GTPases, induce IL-1 β release by the Pyrin inflammasome^{2,3}. *Clostridial* toxins induce glucosylation, adenylation, ADP-ribosylation or deamidation of the switch-I region of RhoA, which prevents guanine nucleotide binding and GTPase activity. Therefore they hypothesize that downstream signaling of RhoA regulates the Pyrin inflammasome. They find that silencing of the effector kinases *Pkn1* and *Pkn2* or chemical PKN inhibition induces IL-1 β release and PKN activation prevents it. It's known that Pyrin is phosphorylated and interacts with 14-3-3 proteins, but the kinase responsible and functional relevance has been elusive⁸. Chae and colleagues now show that PKN1/2 phosphorylate Pyrin on Ser208 and Ser242. Significantly, Ser242Arg mutation also causes Pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND)⁹. PKN1/2-dependent phosphorylation of human Pyrin encoding a B30.2/SPRY domain was markedly reduced and was even further reduced for Pyrin carrying FMF-associated mutations, suggesting that this domain negatively regulates the binding of PKN to- and phosphorylation of Pyrin. Furthermore, binding of 14-3-3 proteins is diminished in the presence of FMF-associated mutations. Hence, 14-3-3 proteins maintain Pyrin in an inactive conformation and loss of phosphorylation and the resulting failure of 14-3-3 protein binding promotes FMF (Fig. 1b). A similar scenario has been identified for PAAND mutations, where 14-3-3 proteins bound only to phosphorylated Pyrin, but not PAAND Pyrin⁹. RhoA geranylgeranylation is necessary for its activation, and the authors therefore investigated the link of Pyrin to MVK and observed that statins cause a dose dependent release of IL-1 β dependent on Pyrin, but independent of NLRP3, NLRC4 and AIM2.

These two groundbreaking reports, although originating from opposite directions, arrive at a similar conclusion by highlighting the importance of prenylation and small GTPase in Pyrin inflammasome activation and by connecting disruptions of this pathway to PAAND, FMF and HIDS. However, some distinctions exist. While Wang and colleagues propose that MVK-dependent prenylation of Kras is required to prevent Pyrin inflammasome activation and IL-1 β release⁴, Chae and colleagues conclude that MVK-dependent geranylgeranylation and other forms of RhoA activation is required to prevent Pyrin inflammasome activation through maintaining phosphorylation of Pyrin and interaction with inhibitory 14-3-3 protein guards (Fig. 1c)³. Hence, Pyrin functions as a sensor of small GTPase activity, which is frequently targeted by bacterial toxins to impair fundamental cellular processes. Depending on which GTPase activity is disrupted, elicited signals modulate expression of *Mefv* and pro-inflammatory mediators or directly regulate phosphorylation and subsequent activity of Pyrin. Hence, synergistic mechanisms that induce Pyrin inflammasome expression and disrupt the 14-3-3 guards on Pyrin to culminate in Pyrin inflammasome activation and IL-1 β release would support a successful bacterial clearance. Due to their high carrier frequency in Mediterranean and Middle Eastern populations there have long been speculations about the possible advantage of FMF-associated *MEFV* mutations. Fine-tuning of Pyrin inflammasome activity may be beneficial under certain circumstances to promote host defense. FMF-linked mutations in B30.2/SPRY reduce or prevent Pyrin phosphorylation and activate Pyrin, likely through altered intramolecular interactions to block interaction with PKN1/2 and subsequently 14-3-3 proteins, contrary to the intramolecular interactions

between the LRRs and the NACHT in NLRs or the HIN200 and PYRIN domain interaction in ALRs, which keep these sensors inactive. However, how mice, which completely lack the B30.2/SPRY domain would then promote Pyrin activation is currently unknown, but the authors speculate that phosphatase(s) may be necessary and their identification will be the next logical step in further elucidating Pyrin inflammasome regulation. Such an innate immune guard-type mechanism is well-established in plant disease resistance R proteins during the hypersensitive response, which involves phosphorylation of negative regulators and plant 14-3-3 proteins have a prominent role in this response^{10,11}. Such effector-triggered immunity of sensing bacterial toxin activity by Pyrin represents a new paradigm in mammalian innate immunity in which innate immune responses are indirectly triggered by a guard-type mechanism to enable detection of broad activities of live pathogenic bacteria. However, defects in any part of this guard system cause the uncontrolled production of IL-1 β and systemic inflammatory disease. Consequently, these new insights may have important clinical implications, as traditionally, FMF and HIDS have not been linked, but placing them into the ‘inflammasomopathies’ may enable the application of the well-established cadre of IL-1 β -targeting drugs. These two studies therefore provide an important piece in the puzzle of understanding mammalian pathogen sensing and how defects in this system cause autoinflammatory disease.

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Figure 1. The guard-type Pyrin inflammasome

(a) Bacterial toxins, such as TcdB, C3, VopS and IbpA or intracellular cAMP cause inactivation of RhoA and consequently disrupt PKN-mediated phosphorylation of Pyrin, which releases the 14-3-3 protein guards from Pyrin to promote assembly of the Pyrin inflammasome and release IL-1 β and IL-18 to support host defense against these bacterial pathogens. (b), Deficiency in the mevalonate kinase (MVK) as in MKD/HIDS patients impairs Kras geranylgeranylation, Kras binding and activation of Akt1 and subsequently causes elevated pro-inflammatory cytokine release and *MEFV* expression. In parallel,

geranylgeranylation-dependent RhoA activation is impaired, which releases the 14-3-3 guard from Pyrin and causes constitutive and chronic Pyrin inflammasome assembly and activation and excessive release of IL-1 β . Similarly, hereditary mutations in Pyrin, as observed in FMF patients, prevent interaction with PKNs and 14-3-3 proteins or directly destroy the serine 242 phosphorylation site, as observed in PAAND patients, resulting in excessive Pyrin inflammasome activation. Hence, temporary disruption of any part of this guard pathway causes Pyrin-dependent host defense responses, while constant disruption by genetic mutations causes IL-1 β -dependent auto-inflammatory disease. (c) During homeostasis, sufficient geranylgeranyl pyrophosphate is generated through the metabolic mevalonate pathway through the activities of MVK and the geranylgeranyl transferase I (GGTase I) to appropriately geranylgeranilate small GTPases, including Kras and RhoA to tether them to the plasma membrane. Active, geranylgeranylated Kras regulates TLR-mediated PI3K/Akt1 activation to balance transcription of pro-inflammatory and anti-inflammatory mediators and downregulates *MEFV* expression. At the same time activation of the RhoA effector kinases PKN1 and PKN2 results in phosphorylation of Pyrin on serine 208 and 242 and enables interaction with members of the 14-3-3 family, which guard activation of Pyrin.