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Summary

Autoinflammatory syndromes are disorders characterized by the hyperactivation of the innate immune system in the absence of microbial infection or autoantibody production. Some autoinflammatory syndromes are associated with recurrent episodes of fever and systemic inflammation that are caused by dysregulated activation of inflammasomes, molecular platforms responsible for the activation of caspase-1 and the production of interleukin (IL)-1β. In this review we will discuss the role of IL-1β and the inflammasomes in host defence and how mutations of two genes, *NLRP3* and *PYRIN*, leads to the autoinflammatory syndromes, cryopyrin-associated periodic syndromes (CAPS) and familial Mediterranean fever (FMF). Both CAPS and FMF are characterized by increased inflammasome activity and overproduction of IL-1β which is ultimately responsible for disease manifestations. Importantly, understanding the molecular mechanisms of these syndromes has led to effective treatment for these rare diseases with biological drugs that target IL-1β-mediated signalling.

Keywords: IL-1β, inflammasome, inflammation, innate immunity

Introduction

The immune system can be categorized broadly as innate and adaptive. Disorders of the immune system may be due to hyperactivation or impaired activation of either the innate or the adaptive immune systems. In the case of deficient activation of the immune system the resultant diseases are called immunodeficiencies, and are characterized phenotypically by recurrent episodes of infection. An exaggerated activation of the adaptive immune system results in the generation of self-reactive lymphocytes and high-titre autoantibodies that are typical features of autoimmune diseases. In contrast, disorders of the innate immune system with little or no involvement of T and B cells are called autoinflammatory syndromes and are characterized by recurrent episodes of fever and systemic inflammation in the absence of microbial infection [1]. For some of these diseases, the gene responsible for the exaggerated activation of the immune system has been identified, and the list of hereditary autoinflammatory syndromes is constantly growing due to the discovery of different genetic mutations that underlie these disorders. Here we will focus on those disorders in which the genetic mutation responsible for the disease that triggers an exaggerated production of interleukin (IL)-1β is due to dysregulation of inflammasomes, and we refer the reader to other excellent reviews for a more comprehensive classification of autoinflammatory syndromes [1–4].

IL-1β

IL-1β is a powerful mediator of inflammatory responses [5]. Systemically, IL-1β induces fever and the hepatic acute phase response that includes C-reactive protein and serum amyloid A, and acts on the bone marrow to promote neutrophilia. At a local level, IL-1β induces expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), P-selectin and E-selectin [6,7], which promote neutrophil recruitment to inflamed tissues [8,9]. In addition, IL-1β acts directly on neutrophils and other leucocyte populations to regulate their activation and production of other mediators of inflammation such as tumour necrosis factor (TNF)-α and IL-6 [8]. These observations suggest that IL-1β has a role in host defence, and indeed there is compelling evidence that mice

deficient in IL-1β are more susceptible than wild-type to infection with bacteria, viruses or fungi [10,11]. Based on the effect of injection of recombinant IL-1β, the concentration of IL-1β that promotes host defence in humans has been estimated to be ~1–10 ng/kg. However, when produced for an excessive period of time, IL-1β causes tissue damage, bone resorption, collagen deposition and neovascularization. Moreover, IL-1β produced in large amounts promotes harmful systemic responses, including hypotension at concentrations higher than 30 ng/kg and haemodynamic shock at concentrations higher then 300 ng/kg. This evidence suggests that the production of IL-1β must be tightly controlled to avoid detrimental effects or even death due to haemodynamic shock [8]. We will discuss first how the production of IL-1β is normally controlled in healthy individuals and then discuss the molecular mechanisms that are involved in the excessive production of IL-1β in selected autoinflammatory syndromes. It is worth mentioning that the symptoms common to all autoinflammatory syndromes, such as episodes of fever, increased concentration of acute phase proteins that can lead to amyloidosis and accumulation of neutrophils at involved sites, can be caused by IL-1β overproduction, which might explain the efficacy of biologicals that target IL-1β in the treatment of these disorders.

Regulation of IL-1β production

The production and action of IL-1β is regulated at different levels: induction of the immature cytokine pro-IL-1β, maturation of pro-IL-1β into the biologically active cytokine IL-1β, secretion of IL-1β and binding of IL-1β to IL-1R on target cells. In monocytes, macrophages and dendritic cells pro-IL-1β is expressed only at very low levels. Several cytokines, including TNF-α, IL-1α and IL-1β, as well as stimulation of phagocytic cells with Toll-like receptor (TLR)-ligands such as lipopolysaccharide (LPS), that signals via TLR-4, or NLR [nucleotide oligomerization domain (NOD)-like receptor]-ligands, such as muramyl dipeptide (MDP) that signals via NOD2, induce activation of the transcription nuclear factor (NF)-κB and the up-regulation of pro-IL-1β [12–14]. Therefore, proinflammatory cytokines and stimulation of the innate immune system are necessary for the production of pro-IL-1β. However, in the absence of a 'secondary stimulus', the maturation of pro-IL-1β is very inefficient. The maturation of pro-IL-1β is mediated by proteolytic processing into 17-kDa mature IL-1β by the cysteine-protease caspase-1. Caspase-1 itself is present in the cytosol as an inactive protein, pro-caspase-1 [15,16]. The activation of caspase-1 is mediated by its autoproteolytic cleavage. In the last decade, we and others have found that the 'secondary stimulus' that induces the production of IL-1β promotes the formation of a molecular platform, generically called inflammasome, which induce the oligomerization and activation of procaspase-1, and will be discussed in more detail later. Once activated, the inflammasome induces the activation of caspase-1 that mediates the maturation of IL-1β [16]. The next step is the secretion of IL-1β in the extracellular environment. IL-1β lacks the leader sequence found generally in secreted proteins, and several non-conventional routes of secretion have been proposed including exosome shedding [17], shedding of plasma membrane microvesicles [18] and lysosomal secretion [19], which might be amplified further by membrane permabilization secondary to necrosis. It is worth mentioning that caspase-1 activation, IL-1β processing and its secretion are highly associated processes [20]. Next, the secreted IL-1β binds to its receptor IL-1R, a process that is inhibited by its natural antagonist IL-1Ra (IL-1F3), a protein that is also secreted in response to many proinflammatory stimuli. IL-1Ra binds to the cell surface IL-1R with higher affinity than IL-1β and prevents IL-1β from signalling. Mice deficient in IL-1Ra develop arthritis [21] and arteritis [22], underscoring the importance of IL-1Ra in regulating the activity of IL-1β. Strikingly, infants born with non-functional IL-1Ra develop a lethal inflammatory disorder, characterized by neutrophil-laden pustular skin eruption, vasculitis and bone abnormalities in the absence of any detectable infection [23,24]. This autoinflammatory syndrome, called DIRA (deficiency in IL-1 receptor antagonist) is currently treated efficiently with a recombinant IL-1Ra, anakinra, that prevents all disease manifestations [23,24].

Inflammasomes

Inflammasomes are molecular platforms responsible for the activation of caspase-1, a cysteine protease that mediates proteolytic processing and activation of the proinflammatory cytokines IL-1β and IL-18 [15,25]. The first evidence for the existence of the inflammasome was described originally in cell extracts of human acute monocytic (THP-1) cells incubated in a buffer containing low levels of K^+ [26]. Evidence accumulated in the last 5 years demonstrated that different physiological stimuli engage different inflammasomes to induce the activation of caspase-1, and these platforms have been named the NLRP1-inflammasome, the NLRP3-inflammasome, the NLRC4-inflammasome and the absent in melanoma 2 (AIM2) inflammasome [15,25,27]. NLRP1, NLRP3 and NLRC4 belong to the NLR family, a family of PRRs that sense pathogen-associated molecular patterns (PAMPs) or endogenous signals of stress in the cytosol. In agreement with their role in sensing cytosolic PAMPs, the mouse Nlrp1b inflammasome is activated anthrax lethal toxin from *Bacillus anthracis*, whereas the human NLRP1 is activated by muramyl dipeptide (MDP) [28]. The NLRC4-inflammasome is activated by cytosolic flagellin from *Salmonella typhimurium* [29,30], *Pseudomonas aeruginosa* [31] and *Legionella pneumophila* [32,33]. Interestingly, the activation of the NLRC4-inflammasome, at least in certain experimental settings, is assisted by neuronal apoptosis inhibitor proteins (NAIPs) [34]. The

NLRC4-inflammasome is also activated by a flagellin-independent pathway in macrophages infected with *Shigella flexneri* [35], and this pathway may be dependent on MxiI, a basal body rod component of the T3SS apparatus [36]. The NLRP3 inflammasome is the most complex and most studied inflammasome and will be discussed separately and in more detail later. AIM2 is a member of the HIN200 protein family and is a cytosolic receptor for double-strand DNA. AIM2 is important for the activation of caspase-1 in response to vaccinia virus, mouse cytomegalovirus (mCMV) and the bacterial pathogen *Francisella tularensis* [37,38]. It is worth mentioning that bacteria can engage multiple inflammasomes. For example, there is evidence that *Listeria monocytogenes* can activate the NLRC4-inflammasome, the NLRP3-inflammasome and the AIM2 inflammasome [39–44]. There is also evidence that inflammasome-independent pathways can induce maturation of IL-1β [45,46].

NLRP3 inflammasome

The NLRP3 inflammasome is the most studied, and most controversial, inflammasome. The NLRP3 inflammasome is unique in that it is the only inflammasome described so far that can be activated by non-microbial stimuli [15,47]. The NLRP3 inflammasome can also be activated by microbes [48].

Activation of the NLRP3-inflammasome requires the combination of two separate signals. 'Signal 1' is necessary to prime macrophages and dendritic cells to a subsequent 'signal 2', that can be considered the true activator of the inflammasome [15,25]. The existence of a 'signal 1' was suggested initially by the observation that adenosine triphosphate (ATP), an activator of the NLRP3-inflammasome, can activate caspase-1 efficiently only in cells pretreated with LPS [49]. We and others found later that in the absence of stimulation with PAMPs several activators of the NLRP3 inflammasome, in addition to ATP, fail to induce caspase-1 activation [50,51]. It was found that PAMPs prime macrophages through a transcriptional event induced by TLR- or NOD-like receptor (NLR)-signalling pathways and mediated via the transcription factor NF-kB [50,51]. Thus, in the case of the NLRP3 inflammasome, NF-κB activation is not only important for the production of pro-IL-1β, but also for activation of the inflammasome. Importantly, Hornung and colleagues found that TLR-signalling induces up-regulation of NLRP3 itself and that in cells over-expressing NLRP3 priming is not necessary for the activation of caspase-1 induced by ATP [50]. These data suggest that one important feature of priming is the up-regulation of NLRP3 itself. It is worth mentioning that in human monocytes, stimulation with PAMPs induces the production of IL-1β in the absence of exogenous stimulation with ATP [52]. Recent studies showed that PAMP stimulation of freshly isolated monocytes promotes the release of endogenous ATP that act in a autocrine fashion on the purinerig receptor P2X7 to promote caspase-1 activation and IL-1β production [53]. These data indicate that the activation of the NLRP3 inflammasomes in human monocytes, mouse macrophages and dendritic cells require two signals.

If priming is necessary for the activation of the NLRP3 inflammasome how can the NLRP3 inflammasome be activated in a sterile environment, where PAMPs are not present? We found that endogenous cytokines, such as TNF-α, IL-1α and IL-1β, can prime macrophages and dendritic cells efficiently for the activation of the NLRP3-inflammasome induced by danger signals and particulate matter [51]. Thus, endogenous cytokines are sufficient to provide 'signal 1' for the activation of the NLRP3-inflammasome. 'Signal 2' for NLRP3 activation can be provided by microbial and non-microbial stimuli. Examples of signal 2 of microbial origin include pore-forming toxins [54,55] and microbial RNA species [56]. Consistently, the NLRP3 inflammasome has been involved in the activation of caspase-1 induced by bacteria [48], viruses [57] and fungi [58]. Examples of NLRP3 activators generated in sterile environments are the danger signal ATP [59], monosodium crystals [60], calcium pyrophosphate dehydrate crystals [60], cholesterol crystals [61] and oligomers of islet amyloid polypeptide (IAPP) [62]. Interestingly, those activators of the NLRP3-inflammasome have been involved in the pathogenesis of diseases such as gout, pseudogout, atherosclerosis and type II diabetes that are diseases that have a significant inflammatory component. One important question, and the reason of much controversy, is whether stimuli of such different nature converge on a common pathway to activate NLRP3. Several theories have been proposed. The first theory is that NLRP3 senses the concentration of cytosolic K^+. Stimuli such as the danger signal ATP that engage the purinergic receptor P2X7 and bacterial pore-forming toxins induce K^+ efflux, thus reducing the cytosolic K^+ concentration [39,41]. In agreement with the role of K^+ in activation of the NLRP3-inflammasome, extracellular medium rich in K^+ that prevent the decrease of cytosolic K^+ concentration block the activation of the NLRP3-inflammasome. The second mechanism proposes that particulate matter induces damage of the lysosome, which is followed by the release of lysosomal molecules, such as cathepsin B, which will be responsible for the activation of NLRP3 [63]. In this respect it is worth mentioning that serine-protease inhibitors can efficiently block the activation of the NLRP3-inflammasome [64]. A third mechanism proposes that reactive oxygen species (ROS) generated by the mitochondria activate TXNIP which, in turn, binds to and activate the NLRP3-inflammasome [65]. It must be noted, however, that the role of TXNIP is controversial in that cells deficient in TXNIP respond normally to stimuli that activate the NLRP3-inflammasome [62]. Moreover, there is evidence that ROS can inhibit caspase-1 activity by glutathionylation of the redox-sensitive cysteine residues

Cys397 and Cys362 [66]. Furthermore, recent evidence indicates that agents commonly used to block ROS generation actually prevent the up-regulation of NLRP3 [67] and pro-IL-1β [68], indicating that they act mainly at the level of signal 1. Consistently, inhibitors of ROS do not block the activation of caspase-1 in cells that express NLRP3 [67].

Cryopyrin-associated periodic syndromes (CAPS)

Familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS) and neonatal onset multi-system inflammatory disease (NOMID) [also called chronic infantile neurologic cutaneous articular syndrome (CINCA)] are different manifestations of one disease, in that they are all caused by autosomal-dominant mutations in NLRP3. Therefore, FCAS, MWS and NOMID are now collectively called cryopyrin-associated periodic syndromes (CAPS) [69,70]. FCAS represents the less severe manifestation of CAPS and is characterized by cold-induced fever and urticaria-like rashes. MWS is more severe, and patients have also sensorineural hearing loss and arthritis. NOMID is the most severe form of CAPS, and patients have epiphyseal overgrowth of the long bones and chronic aseptic meningitis [2]. Mutations that lead to CAPS are clustered inside or in the vicinity of the NOD domain (also referred as the NACHT domain), a module that is thought to be important in promoting the oligomerization of NLR proteins (Fig. 1). Initial experiments indicated that NLRP3 mutations in CAPS are gain-of-function mutations, in that over-expression of disease-associated mutants leads to increased secretion of IL-1β and macrophages from MWS patients secrete more IL-1β than healthy individuals [71,72] (Fig. 1). These early studies uncovered the molecular basis on CAPS and paved the way to the treatment of CAPS with biologicals that target IL-1β-induced signalling. However, the mechanism through which disease-associated mutations led to increased production of IL-1β is still poorly understood. One feature of the NOD domain that is thought to be important to induce the oligomerization of NLRP3 is its ability to bind to and hydrolyze ATP. This activity is required for CAPS-associated mutations to induce IL-1β secretion, suggesting that the gain-of function mutations in NLRP3 do not affect NLRP3 binding to ATP [73]. One possibility is that inhibitory signals that normally act on NLRP3 to repress its function fail to do so if the NOD domain of NLRP3 is mutated. In agreement with this possibility, cytosolic K^+ that normally prevents activation of the NLRP3-inflammasome fails to do so if cells harbour NLRP3-associated mutations [74,75]. One alternative possibility is that disease-associated NLRP3 mutations remove an inhibitory loop, making the NLRP3 variant constitutively active. In agreement with this possibility, stimulation with LPS that induces the up-regulation of NLRP3 is not sufficient to induce the activation of wild-type NLRP3, but is sufficient to induce caspase-1 activation and IL-1β production in macrophages harbouring the NLRP3 mutations associated with CAPS [74,75]. Furthermore, production of IL-1β in monocytes from CAPS patients stimulated with PAMPs is maximal in the absence of ATP stimulation that is required instead to induce maximal production of IL-1β in monocytes from healthy individuals [53]. Recently, a mouse model of CAPS has been developed independently by two groups. Strober *et al.* generated a knock-in mouse harbouring the R258W mutation that corresponds to the R260W substitution found in patients with MWS and FCAS [75]. Hoffman *et al.* generated mouse strains carrying mutations (A350V that corresponds to the A352V variant found in MWS and L351P that corresponds to the L353P variant found in FCAS patients) downstream of a LoxP-flanked neomycin resistance cassette in a reverse orientation [74]. With this strategy the expression of the mutated NLRP3 protein is

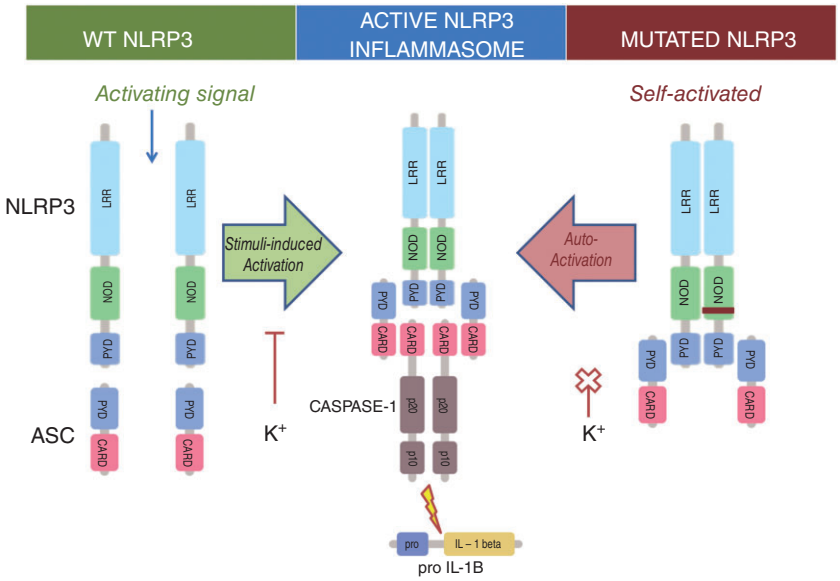

Fig. 1. Right: nucleotide-binding domain and leucine-rich repeat containing family pyrin domain containing 3 (NLRP3) forms an inflammasome only in the presence of activating signals. Left: disease-associated mutations in NLRP3 are gain-of-function mutation located in the nucleotide oligomerization domain (NOD) domain that lead to a constitutive active protein. Cytosolic K^+ inhibits the formation of the NLRP3-inflammasome, but fails to do so in the presence of NLRP3 gain-of-function mutations.

conditional to the expression of Cre-recombinase (Cre), and the authors were able to generate conditional knock-in mice expressing NLRP3 mutant in selected tissues by crossing the mice with mice expressing Cre under different promoters. Although the phenotypes of these mice show different severities of inflammation, all were characterized by the infiltration of neutrophils in the inflamed tissues [74,75]. Furthermore, the inflammatory response was due to the expression of NLRP3 mutants in the myeloid compartment and overproduction of IL-1β [74,75]. Importantly, Hoffman *et al.*'s work showed that disease manifestation is independent of the presence of T cells or B cells, thus providing experimental evidence that autoinflammatory syndromes are primarily disorders of the innate immune system [74]. It must be noted, however, that overproduction of IL-1β led to a T helper type 17 (Th17)-skewed phenotype. Interestingly, anti-IL-17 antibodies were found to ameliorate skin pathology, suggesting that certain disease manifestation may be exacerbated by Th17 cells [75]. This mouse model can help to address some unresolved questions. First, as this disease is not caused by an infection and the cells from these mice do not secrete IL-1β spontaneously, which are the stimuli that are triggering the production of IL-1β? Secondly, as IL-1β is produced by different cells of myeloid origin, such as macrophages, dendritic cells and mast cells, which of these cells (and in which organ) are responsible for the pathological production of IL-1β? Thirdly, why are several organs affected by the disease but others, such as the lung and the intestine, do not show any abnormality? Fourthly, why does the overproduction of IL-1β not predispose to autoimmune responses, when IL-1β is an effective adjuvant?

The important role of IL-1β in CAPS patients is underscored by the efficacy of biologicals that target IL-1β in the treatment of those patients. Recombinant IL-1Ra (anakinra), soluble IL-1 receptor (rilonacept) [76] and a human monoclonal antibody against IL-1β (canakinumab) [77] promote the rapid resolution of symptoms. Remarkably, biologicals that target IL-1β also proved to be effective in ameliorating the neurological symptoms in NOMID patients.

Familial Mediterranean fever (FMF)

FMF is the most prevalent hereditary autoinflammatory syndrome and is caused by mutations in MEFV (MEditerranean FeVer) which encodes for a 781 amino acid protein known as pyrin (or marenostrin) [3]. Human pyrin consists of four domains, an N-terminal pyrin domain (PYD), followed by two B-box zinc-finger and coiled-coil domains and a C-terminal B30·2 domain (also called SPRY). Most of the mutations responsible for FMF are located in the B30·2 domain. FMF is traditionally considered a recessive disorder based on the fact that both alleles are mutated in the majority of patients; however, there is evidence that disease can occur in patients harbouring a single mutated allele and up to 20% of patients that show clinical features of FMF (1–3-day-long sporadic attacks of fever and pain, involving unexplained peritonitis, pleuritis, synovitis, arthritis and erysipelas-like rashes) have no identifiable pyrin mutations [2]. FMF is relatively frequent in eastern Mediterranean populations, and the high rate of carriers harbouring mis-sense mutations suggests that the variants confer a survival advantage [3].

Despite the well-established link between pyrin mutations and FMF, the physiological role of pyrin and how pyrin mutation promotes disease is still controversial, with opposing evidence suggesting that pyrin is a positive or negative regulator of caspase-1 activation. The interpretation that pyrin is a negative regulator of caspase-1 fits with the fact that FMF is a recessive disorder. Initial findings based on the over-expression of pyrin in 293T cells showed that pyrin binds to ASC [78] and limits the activation of the NLRP3-inflammasome [79]. Interestingly, data based on over-expression systems also suggested that the B30·2 domain can bind directly to and inhibit caspase-1 activation, while FMF-associated mutations in the B30·2 domain exhibit reduced binding and inhibition of caspase-1 [79,80]. In agreement with an anti-inflammatory role of pyrin, the down-regulation of pyrin by siRNA in human acute monocytic leukaemia cell line-1 cells resulted in an increased production of IL-1β [80]. Furthermore, mice harbouring a truncated form of pyrin showed reduced response to LPS and were protected from LPS-induced lethality [81]. In contrast, Alnemri's group found that the over-expression of pyrin in 293T cells that stably express ASC and caspase-1 promote the activation of caspase-1 [82]. Moreover, Wewers' group found that the down-regulation of pyrin by siRNA in THP-1 cells or in primary monocytes led to reduced caspase-1 activation and IL-1β [83]. Furthermore, there is evidence that pyrin is involved in the activation of caspase-1 induced by LPS [83], Francisella [84], the retrovirus murine stem cell virus (MSCV) [82] and acts downstream of phosphatase-interacting protein 1 (PSTPIP1) [82], a protein that is mutated in pyogenic arthritis, pyoderma gangrenosum and acne syndrome (PAPA) syndrome, a related autoinflammatory disease. The two opposing models are depicted in Fig. 2. One possible explanation of the different results of those studies is that mouse pyrin differs from that of humans and lacks the B30·2 domain, making it difficult to study the role of pyrin in mouse models. Recently, in an attempt to resolve these controversies, the Kastner group generated a mouse harbouring a chimeric protein of the mouse pyrin fused to the human B30·2 domain (pyrin-mB30·2) [85] and pyrin knock-out mice. Although the expression of the fusion protein containing wild-type B30·2 domain was lethal, somewhat limiting the interpretation of the results, the authors were able to generate mice in which the B30·2 domain harboured the mis-sense mutations found in FMF and reported that homozygous mice develop a spontaneous activation of the inflammasome and a systemic inflammatory response [85]. In contrast, pyrin knock-out mice do not

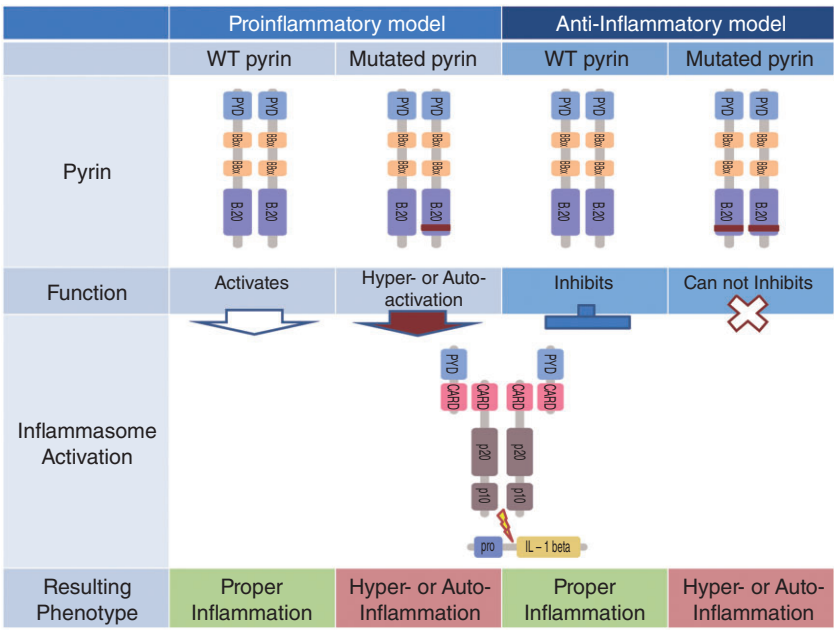

Fig. 2. Two different models have been proposed to explain how mutations in PYRIN lead to familial Mediterranean fever (FMF). In one model pyrin is a positive regulator and promote the activation of caspase-1 in response to specific stimuli. Dominant gain-of-function mutations lead to a constitutive active protein, similarly to what happen for the gain-of-function mutation in nucleotide-binding domain and leucine-rich repeat containing family pyrin domain containing 3 (NLRP3). Alternatively, pyrin is a negative regulator of caspase-1. According to this model, recessive loss-of-function mutations fail to control the activation of caspase-1 leading to the inflammatory response observed in FMF.

show any obvert phenotype, indicating that the functional role of pyrin-mB30·2 and pyrin is different and suggest that pyrin-mB30·2 is a valuable model of FMF. Consistent with FMF caused by genetic lesions of the innate immune system, pyrin is expressed mainly in innate immune cells such as neutrophils, monocytes and dendritic cells, but not in lymphocytes. In an elegant set of experiments, the Kastner group showed that bone-marrow transplantation of donor pyrin-mB30·2 in wild-type recipients induces the development of an inflammatory phenotype that recapitulates the disease manifestation seen in unmanipulated pyrin-mB30·2 knock-in mice [85]. The authors also performed an experiment with bone-marrow transplantation of wild-type haematopoietic cells into recipient pyrin-mB30·2 knock-in mice. As the knock-in mice develop severe symptoms early in life, the authors were forced to adopt a suboptimal protocol of bone-marrow transplantation, but a careful analysis of the efficiency of chimerism in transplanted mice indicate a strong correlation between the efficiency of bone-marrow transplantation and the amelioration of the disease [85]. Furthermore, the authors performed experiments to test the role of adaptive immunity in FMF. To this end, they generated pyrin-mB30·2 knock-in mice in a Rag1-deficient background, which are deficient in T lymphocyte and B lymphocytes, and showed that these mice develop an autoinflammatory syndrome similar to the one in pyrin-mB30·2 knock-in mice. Importantly, by generating pyrin-mB30·2 knock-in mice in a background deficient in inflammasome components, the authors proved that the disease was dependent on caspase-1, ASC and IL-1 signalling but was independent of NLRP3, NLRC4 and AIM2 [85]. As mentioned previously, the disease developed only in homozygous mice, which is consistent with a recessive mode of inheritance, and the authors suggest that pyrin mutations are gain-of-function mutations with a dosage effect in inducing disease. A main question that remains to be addressed is whether pyrin is physiologically an adaptor or forms an inflammasome using the B30·2 domain to sense microbes.

FMF is treated routinely with colchicine, a drug that is known to target microtubules [86]. However, the mechanism of action of colchicine in FMF is not clear, and may be due to either the inhibition of the inflammasome or to the inhibition of the migration of inflammatory cells in inflamed tissues. Nevertheless, some patients are not sensitive, or intolerant, to colchicine. In those patients biologicals that target IL-1β have been shown to be effective [87].

Conclusions and future perspectives

Understanding that gain-of-function mutations in NLRP3, along with the discovery of the role of NLRP3 in activating caspase-1 and IL-1β production, have led to the use of biologicals that target IL-1β signalling to treat disease. Other hereditary syndromes [such as FMF, PAPA syndrome and hyperimmunoglobulinaemia D with periodic fever syndrome (HIDS)] may be due to increased activation of inflammasomes and elevated production of IL-1β. Identification of genes involved in other hereditary autoinflammatory syndromes may lead to a better understanding of the inflammasome and new targets to treat these inflammatory disorders. One of the challenges for the future will be assessing the role of inflammasomes and IL-1β in other hereditary syndromes in which the gene responsible for the disease has not yet been identified, as well as in complex and polygenic conditions characterized by an exaggerated activation of the

innate immune system, such as gout, pseudogout and type 2 diabetes mellitus.

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Disclosure

The authors declare that no conflict of interest exists.

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